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(54) Title: THERAPEUTIC EPITOPES AND USES THEREOF

(57) Abstract: The invention herein disclosed is related to epitopes useful in methods of diagnosing, treating, and preventing coeliac disease. Therapeutic compositions which comprise at least one epitope are provided.

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THERAPEUTIC EPITOPES AND USES THEREOF

The invention relates to epitopes useful in the diagnosis and therapy of coeliac disease, including diagnostics, therapeutics, kits, and methods of using the foregoing.

5 An immune reaction to gliadin (a component of gluten) in the diet causes coeliac disease. It is known that immune responses in the intestinal tissue preferentially respond to gliadin which has been modified by an intestinal transglutaminase. Coeliac disease is diagnosed by detection of anti-endomysial antibodies, but this requires confirmation by the finding of a lymphocytic

10 inflammation in intestinal biopsies. The taking of such a biopsy is inconvenient for the patient.

Investigators have previously assumed that only intestinal T cell responses provide an accurate indication of the immune response against gliadins. Therefore they have concentrated on the investigation of T cell responses in intestinal tissue¹.

15 Gliadin epitopes which require transglutaminase modification (before they are recognised by the immune system) are known².

The inventors have found the immunodominant T cell A-gliadin epitope recognised by the immune system in coeliac disease, and have shown that this is recognised by T cells in the peripheral blood of individuals with coeliac disease (see

20 WO 01/25793). Such T cells were found to be present at high enough frequencies to be detectable without restimulation (i.e. a 'fresh response' detection system could be used). The epitope was identified using a non-T cell cloning based method which provided a more accurate reflection of the epitopes being recognised. The immunodominant epitope requires transglutaminase modification (causing

25 substitution of a particular glutamine to glutamate) before immune system recognition.

Based on this work the inventors have developed a test which can be used to diagnose coeliac disease at an early stage. The test may be carried out on a sample from peripheral blood and therefore an intestinal biopsy is not required. The test is

30 more sensitive than the antibody tests which are currently being used.

The invention thus provides a method of diagnosing coeliac disease, or susceptibility to coeliac disease, in an individual comprising:

(a) contacting a sample from the host with an agent selected from (i) the epitope comprising sequence which is: SEQ ID NO:1 (PQPELPY) or SEQ ID NO:2 (QLQPFPQPELPYPQPQS), or an equivalent sequence from a naturally occurring homologue of the gliadin represented by SEQ ID NO:3, (ii) an epitope comprising

5 sequence comprising: SEQ ID NO:1, or an equivalent sequence from a naturally occurring homologue of the gliadin represented by SEQ ID NO:3 (shown in Table 1), which epitope is an isolated oligopeptide derived from a gliadin protein, (iii) an analogue of (i) or (ii) which is capable of being recognised by a T cell receptor that recognises (i) or (ii), which in the case of a peptide analogue is not more than 50

10 amino acids in length, or (iv) a product comprising two or more agents as defined in (i), (ii) or (iii), and (b) determining *in vitro* whether T cells in the sample recognise the agent, recognition by the T cells indicating that the individual has, or is susceptible to, coeliac disease.

Through comprehensive mapping of wheat gliadin T cell epitopes (see

15 Example 13), the inventors have also found epitopes bioactive in coeliac disease in HLA-DQ2+ patients in other wheat gliadins, having similar core sequences (e.g., SEQ ID NOS:18-22) and similar full length sequences (e.g., SEQ ID NOS:31-36), as well as in rye secalins and barley hordeins (e.g., SEQ ID NOS:39-41); see also Tables 20 and 21. Additionally, several epitopes bioactive in coeliac disease in

20 HLA-DQ8+ patients have been identified (e.g., SEQ ID NOS:42-44, 46). This comprehensive mapping thus provides the dominant epitopes recognized by T cells in coeliac patients. Thus, the above-described method and other methods of the invention described herein may be performed using any of these additional identified epitopes, and analogues and equivalents thereof; (i) and (ii) herein include these

25 additional epitopes. That is, the agents of the invention also include these novel epitopes.

The invention also provides use of the agent for the preparation of a diagnostic means for use in a method of diagnosing coeliac disease, or susceptibility to coeliac disease, in an individual, said method comprising determining whether T

30 cells of the individual recognise the agent, recognition by the T cells indicating that the individual has, or is susceptible to, coeliac disease.

The finding of an immunodominant epitope which is modified by transglutaminase (as well as the additional other epitopes defined herein) also allows diagnosis of coeliac disease based on determining whether other types of immune response to this epitope are present. Thus the invention also provides a method of 5 diagnosing coeliac disease, or susceptibility to coeliac disease, in an individual comprising determining the presence of an antibody that binds to the epitope in a sample from the individual, the presence of the antibody indicating that the individual has, or is susceptible to, coeliac disease.

The invention additionally provides the agent, optionally in association with a 10 carrier, for use in a method of treating or preventing coeliac disease by tolerising T cells which recognise the agent. Also provided is an antagonist of a T cell which has a T cell receptor that recognises (i) or (ii), optionally in association with a carrier, for use in a method of treating or preventing coeliac disease by antagonising such T cells. Additionally provided is the agent or an analogue that binds an antibody (that 15 binds the agent) for use in a method of treating or preventing coeliac disease in an individual by tolerising the individual to prevent the production of such an antibody.

The invention provides a method of determining whether a composition is capable of causing coeliac disease comprising determining whether a protein capable of being modified by a transglutaminase to an oligopeptide sequence as defined 20 above is present in the composition, the presence of the protein indicating that the composition is capable of causing coeliac disease.

The invention also provides a mutant gliadin protein whose wild-type sequence can be modified by a transglutaminase to a sequence that comprises an epitope comprising sequence as defined above, but which mutant gliadin protein has 25 been modified in such a way that it does not contain sequence which can be modified by a transglutaminase to a sequence that comprises such an epitope comprising sequence; or a fragment of such a mutant gliadin protein which is at least 15 amino acids long and which comprises sequence which has been modified in said way.

The invention also provides a protein that comprises a sequence which is able 30 to bind to a T cell receptor, which T cell receptor recognises the agent, and which sequence is able to cause antagonism of a T cell that carries such a T cell receptor.

Additionally the invention provides a food that comprises the proteins defined above.

SUMMARY OF THE INVENTION

- 5 The present invention provides methods of preventing or treating coeliac disease comprising administering to an individual at least one agent selected from: a) a peptide comprising at least one epitope comprising a sequence selected from the group consisting of SEQ ID NOs:18-22, 31-36, 39-44, and 46, and equivalents thereof; and b) an analogue of a) which is capable of being recognised by a T cell
- 10 receptor that recognises the peptide of a) and which is not more than 50 amino acids in length; and c) optionally, in addition to the agent selected from a) and b), a peptide comprising at least one epitope comprising a sequence selected from SEQ ID NO:1 and SEQ ID NO:2. In some embodiments, the agent is HLA-DQ2-restricted, HLA-DQ8-restricted or one agent is HLA-DQ2-restricted and a second agent is HLA-DQ8-restricted. In some embodiments, the agent comprises a wheat epitope, a rye
- 15 epitope, a barley epitope or any combination thereof either as a single agent or as multiple agents.

The present invention also provides methods of preventing or treating coeliac disease comprising administering to an individual a pharmaceutical composition comprising an agent above and pharmaceutically acceptable carrier or diluent.

The present invention also provides methods of preventing or treating coeliac disease comprising administering to an individual a pharmaceutical composition comprising an antagonist of a T cell which has a T cell receptor as defined above, and a pharmaceutically acceptable carrier or diluent.

25 The present invention also provides methods of preventing or treating coeliac disease comprising administering to an individual a composition for tolerising an individual to a gliadin protein to suppress the production of a T cell or antibody response to an agent as defined above, which composition comprises an agent as defined above.

30 The present invention also provides methods of preventing or treating coeliac disease by 1) diagnosing coeliac disease in an individual by either: a) contacting a sample from the host with at least one agent selected from: i) a peptide comprising at

least one epitope comprising a sequence selected from the group consisting of: SEQ ID NOS:18-22, 31-36, 39-44, and 46, and equivalents thereof; and ii) an analogue of i) which is capable of being recognised by a T cell receptor that recognises i) and which is not more than 50 amino acids in length; and iii) optionally, in addition to the agent selected from i) and ii), a peptide comprising at least one epitope comprising a sequence selected from SEQ ID NOS:1 and 2; and determining *in vitro* whether T cells in the sample recognise the agent; recognition by the T cells indicating that the individual has, or is susceptible to, coeliac disease; or b) administering an agent as defined above and determining *in vivo* whether T cells in the individual recognise the agent, recognition of the agent indicating that the individual has or is susceptible to coeliac disease; and 2) administering to an individual diagnosed as having, or being susceptible to, coeliac disease a therapeutic agent for preventing or treating coeliac disease.

The present invention also provides agents as defined above, optionally in association with a carrier, for use in a method of treating or preventing coeliac disease by tolerising T cells which recognise the agent.

The present invention also provides antagonists of a T cell which has a T cell receptor as defined above, optionally in association with a carrier, for use in a method of treating or preventing coeliac disease by antagonising such T cells.

The present invention also provides proteins that comprises a sequence which is able to bind to a T cell receptor, which T cell receptor recognises an agent as defined above, and which sequence is able to cause antagonism of a T cell that carries such a T cell receptor.

The present invention also provides pharmaceutical compositions comprising an agent or antagonist as defined and a pharmaceutically acceptable carrier or diluent.

The present invention also provides compositions for tolerising an individual to a gliadin protein to suppress the production of a T cell or antibody response to an agent as defined above, which composition comprises an agent as defined above.

The present invention also provides compositions for antagonising a T cell response to an agent as defined above, which composition comprises an antagonist as defined above.

The present invention also provides mutant gliadin proteins whose wild-type sequence can be modified by a transglutaminase to a sequence which is an agent as defined in claim 1, which mutant gliadin protein comprises a mutation which prevents its modification by a transglutaminase to a sequence which is an agent as defined above; or a fragment of such a mutant gliadin protein which is at least 15 amino acids long and which comprises the mutation.

5 The present invention also provides polynucleotides that comprises a coding sequence that encodes a protein or fragment as defined above.

10 The present invention also provides cells comprising a polynucleotide as defined above or which has been transformed with such a polynucleotide.

The present invention also provides mammals that expresses a T cell receptor as defined above.

15 The present invention also provides methods of diagnosing coeliac disease, or susceptibility to coeliac disease, in an individual comprising: a) contacting a sample from the host with at least one agent selected from i) a peptide comprising at least one epitope comprising a sequence selected from the group consisting of: SEQ ID NOS:18-22, 31-36, 39-44, and 46, and equivalents thereof; and ii) an analogue of i) which is capable of being recognised by a T cell receptor that recognises i) and which is not more than 50 amino acids in length; and iii) optionally, in addition to the agent selected from i) and ii), a peptide comprising at least one epitope comprising a sequence selected from SEQ ID NOS:1 and 2; and b) determining *in vitro* whether T cells in the sample recognise the agent; recognition by the T cells indicating that the individual has, or is susceptible to, coeliac disease.

20

25 The present invention also provides methods of determining whether a composition is capable of causing coeliac disease comprising determining whether a protein capable of being modified by a transglutaminase to an oligopeptide sequence is present in the composition, the presence of the protein indicating that the composition is capable of causing coeliac disease.

30 The present invention also provides methods of identifying an antagonist of a T cell, which T cell recognises an agent as defined above, comprising contacting a candidate substance with the T cell and detecting whether the substance causes a decrease in the ability of the T cell to undergo an antigen specific response, the

detecting of any such decrease in said ability indicating that the substance is an antagonist.

The present invention also provides kits for carrying out any of the method described above comprising an agent as defined above and a means to detect the 5 recognition of the peptide by the T cell.

The present invention also provides methods of identifying a product which is therapeutic for coeliac disease comprising administering a candidate substance to a mammal as defined above which has, or which is susceptible to, coeliac disease and determining whether substance prevents or treats coeliac disease in the mammal, the 10 prevention or treatment of coeliac disease indicating that the substance is a therapeutic product.

The present invention also provides processes for the production of a protein encoded by a coding sequence as defined above which process comprises: a) cultivating a cell described above under conditions that allow the expression of the 15 protein; and optionally b) recovering the expressed protein.

The present invention also provides methods of obtaining a transgenic plant cell comprising transforming a plant cell with a vector as described above to give a transgenic plant cell.

The present invention also provides methods of obtaining a first-generation 20 transgenic plant comprising regenerating a transgenic plant cell transformed with a vector as described above to give a transgenic plant.

The present invention also provides methods of obtaining a transgenic plant seed comprising obtaining a transgenic seed from a transgenic plant obtainable as described above.

25 The present invention also provides methods of obtaining a transgenic progeny plant comprising obtaining a second-generation transgenic progeny plant from a first-generation transgenic plant obtainable by a method as described above, and optionally obtaining transgenic plants of one or more further generations from the second-generation progeny plant thus obtained.

30 The present invention also provides transgenic plant cells, plants, plant seeds or progeny plants obtainable by any of the methods described above.

The present invention also provides transgenic plants or plant seeds comprising plant cells as described above.

The present invention also provides transgenic plant cell calluses comprising plant cells as described above obtainable from a transgenic plant cell, first-generation 5 plant, plant seed or progeny as defined above.

The present invention also provides methods of obtaining a crop product comprising harvesting a crop product from a plant according to any method described above and optionally further processing the harvested product.

The present invention also provides food that comprises a protein as defined 10 above.

BRIEF DESCRIPTION OF THE DRAWINGS

The invention is illustrated by the accompanying drawings in which:

Figure 1 shows freshly isolated PBMC (peripheral blood mononuclear cell)

15 IFN γ ELISPOT responses (vertical axis shows spot forming cells per 10^6 PBMC) to transglutaminase (tTG)-treated and untreated peptide pool 3 (each peptide 10 μ g/ml) including five overlapping 15mers spanning A-gliadin 51-85 (see Table 1) and a-chymotrypsin-digested gliadin (40 μ g/ml) in coeliac disease Subject 1, initially in remission following a gluten free diet then challenged with 200g bread daily for three 20 days from day 1 (a). PBMC IFN γ ELISPOT responses by Subject 2 to tTG-treated A-gliadin peptide pools 1-10 spanning the complete A-gliadin protein during ten day bread challenge (b). The horizontal axis shows days after commencing bread.

Figure 2 shows PBMC IFN γ ELISPOT responses to tTG-treated peptide pool 3 (spanning A-gliadin 51-85) in 7 individual coeliac disease subjects (vertical axis 25 shows spot forming cells per 10^6 PBMC), initially in remission on gluten free diet, challenged with bread for three days (days 1 to 3). The horizontal axis shows days after commencing bread. (a). PBMC IFN γ ELISPOT responses to tTG-treated overlapping 15mer peptides included in pool 3; bars represent the mean (\pm SEM) response to individual peptides (10 μ g/ml) in 6 Coeliac disease subjects on day 6 or 30 7(b). (In individual subjects, ELISPOT responses to peptides were calculated as a % of response elicited by peptide 12 - as shown by the vertical axis.)

Figure 3 shows PBMC IFN γ ELISPOT responses to tTG-treated truncations of A-gliadin 56-75 (0.1 μ M). Bars represent the mean (\pm SEM) in 5 Coeliac disease subjects. (In individual subjects, responses were calculated as the % of the maximal response elicited by any of the peptides tested.)

Figure 4 shows how the minimal structure of the dominant A-gliadin epitope was mapped using tTG-treated 7-17mer A-gliadin peptides (0.1 μ M) including the sequence, PQPQLPY (SEQ ID NO:4) (A-gliadin 62-68) (a), and the same peptides without tTG treatment but with the substitution Q→E65 (b). Each line represents PBMC IFN γ ELISPOT responses in each of three Coeliac disease subjects on day 6 or 7 after bread was ingested on days 1-3. (In individual subjects, ELISPOT responses were calculated as a % of the response elicited by the 17mer, A-gliadin 57-73.)

Figure 5 shows the amino acids that were deamidated by tTG. A-gliadin 56-75 LQLQPFPQPQLPYPQPQSFP (SEQ ID NO:5) (0.1 μ M) was incubated with tTG (50 μ g/ml) at 37°C for 2 hours. A single product was identified and purified by reverse phase HPLC. Amino acid analysis allowed % deamidation (Q→E) of each Gln residue in A-gliadin 56-75 attributable to tTG to be calculated (vertical axis).

Figure 6 shows the effect of substituting Q→E in A-gliadin 57-73 at other positions in addition to Q65 using the 17mers: QLQPFPQPELPYPQPES (SEQ ID NO:6) (E57,65), QLQPFPQPELPYPQPES (SEQ ID NO:7) (E65,72), ELQPFPQPELPYPQPES (SEQ ID NO:8) (E57, 65, 72), and QLQPFPQPELPYPQPQS (SEQ ID NO:2) (E65) in three Coeliac disease subjects on day 6 or 7 after bread was ingested on days 1-3. Vertical axis shows % of the E65 response.

Figure 7 shows that tTG treated A-gliadin 56-75 (0.1 μ M) elicited IFN- γ ELISPOT responses in (a) CD4 and CD8 magnetic bead depleted PBMC. (Bars represent CD4 depleted PBMC responses as a % of CD8 depleted PBMC responses; spot forming cells per million CD8 depleted PBMC were: Subject 4: 29, and Subject 6: 535). (b) PBMC IFN γ ELISPOT responses (spot forming cells/million PBMC) after incubation with monoclonal antibodies to HLA-DR (L243), -DQ (L2) and -DP (B7.21) (10 μ g/ml) 1h prior to tTG-treated 56-75 (0.1 μ M) in two coeliac disease subjects homozygous for HLA-DQ a1*0501, b1*0201.

Figure 8 shows the effect of substituting Glu at position 65 for other amino acids in the immunodominant epitope. The vertical axis shows the % response in the 3 subjects in relation to the immunodominant epitope.

Figure 9 shows the immunoreactivity of naturally occurring gliadin peptides (measuring responses from 3 subjects) which contain the sequence PQLPY (SEQ ID NO:12) with (shaded) and without (clear) transglutaminase treatment.

Figure 10 shows CD8, CD4, β_7 , and α^B -specific immunomagnetic bead depletion of peripheral blood mononuclear cells from two coeliac subjects 6 days after commencing gluten challenge followed by interferon gamma ELISpot. A-gliadin 57-73 QE65 (25mcg/ml), tTG-treated chymotrypsin-digested gliadin (100 mcg/ml) or PPD (10 mcg/ml) were used as antigen.

Figure 11 shows the optimal T cell epitope length.

Figure 12 shows a comparison of A-gliadin 57-73 QE65 with other peptides in a dose response study.

Figure 13 shows a comparison of gliadin and A-gliadin 57-73 QE65 specific responses.

Figure 14 shows the bioactivity of gliadin polymorphisms in coeliac subjects.

Figures 15 and 16 show the defining of the core epitope sequence.

Figures 17 to 27 show the agonist activity of A-gliadin 57-73 QE65 variants.

Figure 28 shows responses in different patient groups.

Figure 29 shows bioactivity of prolamin homologues of A-gliadin 57-73.

Figure 30 shows, for healthy HLA-DQ2 subjects, the change in IFN-gamma ELISpot responses to tTG-deamidated gliadin peptide pools.

Figure 31 shows, for coeliac HLA-DQ2 subjects, the change in IFN-gamma ELISpot responses to tTG-deamidated gliadin peptide pools.

Figure 32 shows individual peptide contributions to "summed" gliadin peptide response.

Figure 33 shows, for coeliac HLA-DQ2/8 subject C08, gluten challenge induced IFN γ ELISpot responses to tTG-deamidated gliadin peptide pools.

Figure 34 shows, for coeliac HLA-DQ2/8 subject C07, gluten challenge induced IFN γ ELISpot responses to tTG-deamidated gliadin peptide pools.

Figure 35 shows, for coeliac HLA-DQ8/7 subject C12, gluten challenge induced IFN γ ELISpot responses to tTG-deamidated gliadin peptide pools.

Figure 36 shows, for coeliac HLA-DQ6/8 subject C11, gluten challenge induced IFN γ ELISpot responses to tTG-deamidated gliadin peptide pools.

5

Detailed Description of the Invention

The term "coeliac disease" encompasses a spectrum of conditions caused by varying degrees of gluten sensitivity, including a severe form characterised by a flat small intestinal mucosa (hyperplastic villous atrophy) and other forms characterised by milder symptoms.

10 The individual mentioned above (in the context of diagnosis or therapy) is human. They may have coeliac disease (symptomatic or asymptomatic) or be suspected of having it. They may be on a gluten free diet. They may be in an acute phase response (for example they may have coeliac disease, but have only ingested 15 gluten in the last 24 hours before which they had been on a gluten free diet for 14 to 28 days).

15 The individual may be susceptible to coeliac disease, such as a genetic susceptibility (determined for example by the individual having relatives with coeliac disease or possessing genes which cause predisposition to coeliac disease).

20

The agent

The agent is typically a peptide, for example of length 7 to 50 amino acids, such as 10 to 40, or 15 to 30 amino acids in length.

SEQ ID NO:1 is PQPELPY. SEQ ID NO:2 is QLQPFPQPELPYPQPQSQ.

25 SEQ ID NO:3 is shown in Table 1 and is the sequence of a whole A-gliadin. The glutamate at position 4 of SEQ ID NO:1 (equivalent to position 9 of SEQ ID NO:2) is generated by transglutaminase treatment of A-gliadin.

30 The agent may be the peptide represented by SEQ ID NO:1 or 2 or an epitope comprising sequence that comprises SEQ ID NO:1 which is an isolated oligopeptide derived from a gliadin protein; or an equivalent of these sequences from a naturally occurring gliadin protein which is a homologue of SEQ ID NO:3. Thus the epitope may be a derivative of the protein represented by SEQ ID NO:3. Such a derivative is

typically a fragment of the gliadin, or a mutated derivative of the whole protein or fragment. Therefore the epitope of the invention does not include this naturally occurring whole gliadin protein, and does not include other whole naturally occurring gliadins.

5 The epitope may thus be a fragment of A-gliadin (e.g. SEQ ID NO:3), which comprises the sequence of SEQ ID NO:1, obtainable by treating (fully or partially) with transglutaminase, i.e. with 1, 2, 3 or more glutamines substituted to glutamates (including the substitution within SEQ ID NO:1).

Such fragments may be or may include the sequences represented by
10 positions 55 to 70, 58 to 73, 61 to 77 of SEQ ID NO:3 shown in Table 1. Typically such fragments will be recognised by T cells to at least the same extent that the peptides represented by SEQ ID NO:1 or 2 are recognised in any of the assays described herein using samples from coeliac disease patients.

Additionally, the agent may be the peptide represented by any of SEQ ID
15 NOS:18-22, 31-36, 39-44, and 46 or a protein comprising a sequence corresponding to any of SEQ ID NOS:18-22, 31-36, 39-44, and 46 (such as fragments of a gliadin comprising any of SEQ ID NOS:18-22, 31-36, 39-44, and 46, for example after the gliadin has been treated with transglutaminase). Bioactive fragments of such sequences are also agents of the invention. Sequences equivalent to any of SEQ ID
20 NOS:18-22, 31-36, 39-44, and 46 or analogues of these sequences are also agents of the invention.

In the case where the epitope comprises a sequence equivalent to the above epitopes (including fragments) from another gliadin protein (e.g. any of the gliadin proteins mentioned herein or any gliadins which cause coeliac disease), such
25 equivalent sequences will correspond to a fragment of a gliadin protein typically treated (partially or fully) with transglutaminase. Such equivalent peptides can be determined by aligning the sequences of other gliadin proteins with the gliadin from which the original epitope derives, such as with SEQ ID NO:3 (for example using any of the programs mentioned herein). Transglutaminase is commercially available
30 (e.g. Sigma T-5398). Table 4 provides a few examples of suitable equivalent sequences.

The agent which is an analogue is capable of being recognised by a TCR which recognises (i) or (ii). Therefore generally when the analogue is added to T cells in the presence of (i) or (ii), typically also in the presence of an antigen presenting cell (APC) (such as any of the APCs mentioned herein), the analogue 5 inhibits the recognition of (i) or (ii), i.e. the analogue is able to compete with (i) or (ii) in such a system.

The analogue may be one which is capable of binding the TCR which recognises (i) or (ii). Such binding can be tested by standard techniques. Such TCRs can be isolated from T cells which have been shown to recognise (i) or (ii) (e.g. using 10 the method of the invention). Demonstration of the binding of the analogue to the TCRs can then be shown by determining whether the TCRs inhibit the binding of the analogue to a substance that binds the analogue, e.g. an antibody to the analogue. Typically the analogue is bound to a class II MHC molecule (e.g. HLA-DQ2) in such an inhibition of binding assay.

15 Typically the analogue inhibits the binding of (i) or (ii) to a TCR. In this case the amount of (i) or (ii) which can bind the TCR in the presence of the analogue is decreased. This is because the analogue is able to bind the TCR and therefore competes with (i) or (ii) for binding to the TCR.

20 T cells for use in the above binding experiments can be isolated from patients with coeliac disease, for example with the aid of the method of the invention.

Other binding characteristics of the analogue may also be the same as (i) or (ii), and thus typically the analogue binds to the same MHC class II molecule to which the peptide binds (HLA-DQ2 or -DQ8). The analogue typically binds to 25 antibodies specific for (i) or (ii), and thus inhibits binding of (i) or (ii) to such antibodies.

The analogue is typically a peptide. It may have homology with (i) or (ii), typically at least 70% homology, preferably at least 80, 90%, 95%, 97% or 99% homology with (i) or (ii), for example over a region of at least 15 more (such as the 30 entire length of the analogue and/or (i) or (ii), or across the region which contacts the TCR or binds the MHC molecule) contiguous amino acids. Methods of measuring protein homology are well known in the art and it will be understood by those of skill

in the art that in the present context, homology is calculated on the basis of amino acid identity (sometimes referred to as "hard homology").

For example the UWGCG Package provides the BESTFIT program which can be used to calculate homology (for example used on its default settings)

5 (Devereux *et al* (1984) *Nucleic Acids Research* 12, p387-395). The PILEUP and BLAST algorithms can be used to calculate homology or line up sequences (typically on their default settings), for example as described in Altschul S. F. (1993) *J Mol Evol* 36:290-300; Altschul, S, F *et al* (1990) *J Mol Biol* 215:403-10.

Software for performing BLAST analyses is publicly available through the

10 National Center for Biotechnology Information on the world wide web through the internet at, for example, "www.ncbi.nlm.nih.gov". This algorithm involves first identifying high-scoring sequence pair (HSPs) by identifying short words of length W in the query sequence that either match or satisfy some positive-valued threshold score T when aligned with a word of the same length in a database sequence. T is referred to as the neighbourhood word score threshold (Altschul *et al*, *supra*). These initial neighbourhood word hits act as seeds for initiating searches to find HSPs containing them. The word hits are extended in both directions along each sequence for as far as the cumulative alignment score can be increased. Extensions for the word hits in each direction are halted when: the cumulative alignment score falls off 20 by the quantity X from its maximum achieved value; the cumulative score goes to zero or below, due to the accumulation of one or more negative-scoring residue alignments; or the end of either sequence is reached. The BLAST algorithm parameters W, T and X determine the sensitivity and speed of the alignment. The BLAST program uses as defaults a word length (W) of 11, the BLOSUM62 scoring 25 matrix (see Henikoff and Henikoff (1992) *Proc. Natl. Acad. Sci. USA* 89: 10915-10919) alignments (B) of 50, expectation (E) of 10, M=5, N=4, and a comparison of both strands.

The BLAST algorithm performs a statistical analysis of the similarity between two sequences; see e.g., Karlin and Altschul (1993) *Proc. Natl. Acad. Sci. USA* 90: 5873-5787. One measure of similarity provided by the BLAST algorithm is the smallest sum probability (P(N)), which provides an indication of the probability by which a match between two nucleotide or amino acid sequences

would occur by chance. For example, a sequence is considered similar to another sequence if the smallest sum probability in comparison of the first sequence to the second sequence is less than about 1, preferably less than about 0.1, more preferably less than about 0.01, and most preferably less than about 0.001.

5 The homologous peptide analogues typically differ from (i) or (ii) by 1, 2, 3, 4, 5, 6, 7, 8 or more mutations (which may be substitutions, deletions or insertions). These mutations may be measured across any of the regions mentioned above in relation to calculating homology. The substitutions are preferably 'conservative'. These are defined according to the following Table. Amino acids in the same block

10 in the second column and preferably in the same line in the third column may be substituted for each other:

ALIPHATIC	Non-polar	G A P
		I L V
	Polar – uncharged	C S T M
		N Q
	Polar – charged	D E
		K R
AROMATIC		H F W Y

15 Typically the amino acids in the analogue at the equivalent positions to amino acids in (i) or (ii) that contribute to binding the MHC molecule or are responsible for the recognition by the TCR, are the same or are conserved.

Typically the analogue peptide comprises one or more modifications, which may be natural post-translation modifications or artificial modifications. The modification may provide a chemical moiety (typically by substitution of a

20 hydrogen, e.g. of a C-H bond), such as an amino, acetyl, hydroxy or halogen (e.g. fluorine) group or carbohydrate group. Typically the modification is present on the N or C terminus.

The analogue may comprise one or more non-natural amino acids, for example amino acids with a side chain different from natural amino acids.

Generally, the non-natural amino acid will have an N terminus and/or a C terminus.

The non-natural amino acid may be an L- or a D- amino acid.

The analogue typically has a shape, size, flexibility or electronic configuration that is substantially similar to (i) or (ii). It is typically a derivative of 5 (i) or (ii). In one embodiment the analogue is a fusion protein comprising the sequence of SEQ ID NO:1 or 2, or any of the other peptides mentioned herein; and non-gliadin sequence.

In one embodiment the analogue is or mimics (i) or (ii) bound to a MHC class II molecule. 2, 3, 4 or more of such complexes may be associated or bound to each 10 other, for example using a biotin/streptavidin based system, in which typically 2, 3 or 4 biotin labelled MHC molecules bind to a streptavidin moiety. This analogue typically inhibits the binding of the (i) or (ii)/MHC Class II complex to a TCR or antibody which is specific for the complex.

The analogue is typically an antibody or a fragment of an antibody, such as a 15 Fab or (Fab)₂ fragment. The analogue may be immobilised on a solid support, particularly an analogue that mimics peptide bound to a MHC molecule.

The analogue is typically designed by computational means and then 20 synthesised using methods known in the art. Alternatively the analogue can be selected from a library of compounds. The library may be a combinatorial library or a display library, such as a phage display library. The library of compounds may be expressed in the display library in the form of being bound to a MHC class II molecule, such as HLA-DQ2 or -DQ8. Analogues are generally selected from the library based on their ability to mimic the binding characteristics (i) or (ii). Thus 25 they may be selected based on ability to bind a TCR or antibody which recognises (i) or (ii).

Typically analogues will be recognised by T cells to at least the same extent 30 as any of the agents (i) or (ii), for example at least to the same extent as the equivalent epitope and preferably to the same extent as the peptide represented by SEQ ID NO:2, is recognised in any of the assays described herein, typically using T cells from coeliac disease patients. Analogues may be recognised to these extents *in vivo* and thus may be able to induce coeliac disease symptoms to at least the same

extent as any of the agents mentioned herein (e.g. in a human patient or animal model).

Analogues may be identified in a method comprising determining whether a candidate substance is recognised by a T cell receptor that recognises an epitope of the invention, recognition of the substance indicating that the substance is an analogue. Such TCRs may be any of the TCRs mentioned herein, and may be present on T cells. Any suitable assay mentioned herein can be used to identify the analogue. In one embodiment this method is carried out *in vivo*. As mentioned above preferred analogues are recognised to at least the same extent as the peptide SEQ ID NO:2, and so the method may be used to identify analogues which are recognised to this extent.

In one embodiment the method comprises determining whether a candidate substance is able to inhibit the recognition of an epitope of the invention, inhibition of recognition indicating that the substance is an analogue.

The agent may be a product comprising at least 2, 5, 10 or 20 agents as defined by (i), (ii) or (iii). Typically the composition comprises epitopes of the invention (or equivalent analogues) from different gliadins, such as any of the species or variety of or types of gliadin mentioned herein. Preferred compositions comprise at least one epitope of the invention, or equivalent analogue, from all of the gliadins present in any of the species or variety mentioned herein, or from 2, 3, 4 or more of the species mentioned herein (such as from the panel of species consisting of wheat, rye, barley, oats and triticale). Thus, the agent may be monovalent or multivalent.

25 Diagnosis

As mentioned above the method of diagnosis of the invention may be based on the detection of T cells that bind the agent or on the detection of antibodies that recognise the agent.

The T cells that recognise the agent in the method (which includes the use mentioned above) are generally T cells that have been pre-sensitised *in vivo* to gliadin. As mentioned above such antigen-experienced T cells have been found to be present in the peripheral blood.

In the method the T cells can be contacted with the agent *in vitro* or *in vivo*, and determining whether the T cells recognise the agent can be performed *in vitro* or *in vivo*. Thus the invention provides the agent for use in a method of diagnosis practiced on the human body. Different agents are provided for simultaneous, 5 separate or sequential use in such a method.

The *in vitro* method is typically carried out in aqueous solution into which the agent is added. The solution will also comprise the T cells (and in certain embodiments the APCs discussed below). The term 'contacting' as used herein includes adding the particular substance to the solution.

10 Determination of whether the T cells recognise the agent is generally accomplished by detecting a change in the state of the T cells in the presence of the agent or determining whether the T cells bind the agent. The change in state is generally caused by antigen specific functional activity of the T cell after the TCR binds the agent. The change of state may be measured inside (e.g. change in 15 intracellular expression of proteins) or outside (e.g. detection of secreted substances) the T cells.

The change in state of the T cell may be the start of or increase in secretion of a substance from the T cell, such as a cytokine, especially IFN- γ , IL-2 or TNF- α . Determination of IFN- γ secretion is particularly preferred. The substance can 20 typically be detected by allowing it to bind to a specific binding agent and then measuring the presence of the specific binding agent/substance complex. The specific binding agent is typically an antibody, such as polyclonal or monoclonal antibodies. Antibodies to cytokines are commercially available, or can be made using standard techniques.

25 Typically the specific binding agent is immobilised on a solid support. After the substance is allowed to bind the solid support can optionally be washed to remove material which is not specifically bound to the agent. The agent/substance complex may be detected by using a second binding agent that will bind the complex. Typically the second agent binds the substance at a site which is different 30 from the site which binds the first agent. The second agent is preferably an antibody and is labelled directly or indirectly by a detectable label.

Thus the second agent may be detected by a third agent that is typically labelled directly or indirectly by a detectable label. For example the second agent may comprise a biotin moiety, allowing detection by a third agent which comprises a streptavidin moiety and typically alkaline phosphatase as a detectable label.

5 In one embodiment the detection system which is used is the *ex-vivo* ELISPOT assay described in WO 98/23960. In that assay IFN- γ secreted from the T cell is bound by a first IFN- γ specific antibody that is immobilised on a solid support. The bound IFN- γ is then detected using a second IFN- γ specific antibody which is labelled with a detectable label. Such a labelled antibody can be obtained from

10 MABTECH (Stockholm, Sweden). Other detectable labels which can be used are discussed below.

15 The change in state of the T cell that can be measured may be the increase in the uptake of substances by the T cell, such as the uptake of thymidine. The change in state may be an increase in the size of the T cells, or proliferation of the T cells, or a change in cell surface markers on the T cell.

20 In one embodiment the change of state is detected by measuring the change in the intracellular expression of proteins, for example the increase in intracellular expression of any of the cytokines mentioned above. Such intracellular changes may be detected by contacting the inside of the T cell with a moiety that binds the expressed proteins in a specific manner and which allows sorting of the T cells by flow cytometry.

25 In one embodiment when binding the TCR the agent is bound to an MHC class II molecule (typically HLA-DQ2 or -DQ8), which is typically present on the surface of an antigen presenting cell (APC). However as mentioned herein other agents can bind a TCR without the need to also bind an MHC molecule.

30 Generally the T cells which are contacted in the method are taken from the individual in a blood sample, although other types of samples which contain T cells can be used. The sample may be added directly to the assay or may be processed first. Typically the processing may comprise diluting of the sample, for example with water or buffer. Typically the sample is diluted from 1.5 to 100 fold, for example 2 to 50 or 5 to 10 fold.

The processing may comprise separation of components of the sample.

Typically mononuclear cells (MCs) are separated from the samples. The MCs will comprise the T cells and APCs. Thus in the method the APCs present in the separated MCs can present the peptide to the T cells. In another embodiment only T 5 cells, such as only CD4 T cells, can be purified from the sample. PBMCs, MCs and T cells can be separated from the sample using techniques known in the art, such as those described in Lalvani *et al* (1997) *J. Exp. Med.* 186, p859-865.

In one embodiment, the T cells used in the assay are in the form of unprocessed or diluted samples, or are freshly isolated T cells (such as in the form of 10 freshly isolated MCs or PBMCs) which are used directly *ex vivo*, i.e. they are not cultured before being used in the method. Thus the T cells have not been restimulated in an antigen specific manner *in vitro*. However the T cells can be cultured before use, for example in the presence of one or more of the agents, and generally also exogenous growth promoting cytokines. During culturing the agent(s) 15 are typically present on the surface of APCs, such as the APC used in the method. Pre-culturing of the T cells may lead to an increase in the sensitivity of the method. Thus the T cells can be converted into cell lines, such as short term cell lines (for example as described in Ota *et al* (1990) *Nature* 346, p183-187).

The APC that is typically present in the method may be from the same 20 individual as the T cell or from a different host. The APC may be a naturally occurring APC or an artificial APC. The APC is a cell that is capable of presenting the peptide to a T cell. It is typically a B cell, dendritic cell or macrophage. It is typically separated from the same sample as the T cell and is typically co-purified with the T cell. Thus the APC may be present in MCs or PBMCs. The APC is 25 typically a freshly isolated *ex vivo* cell or a cultured cell. It may be in the form of a cell line, such as a short term or immortalised cell line. The APC may express empty MHC class II molecules on its surface.

In the method one or more (different) agents may be used. Typically the T 30 cells derived from the sample can be placed into an assay with all the agents which it is intended to test or the T cells can be divided and placed into separate assays each of which contain one or more of the agents.

The invention also provides the agents such as two or more of any of the agents mentioned herein (e.g. the combinations of agents which are present in the composition agent discussed above) for simultaneous separate or sequential use (eg. for *in vivo* use).

5 In one embodiment agent *per se* is added directly to an assay comprising T cells and APCs. As discussed above the T cells and APCs in such an assay could be in the form of MCs. When agents that can be recognised by the T cell without the need for presentation by APCs are used then APCs are not required. Analogues which mimic the original (i) or (ii) bound to a MHC molecule are an example of such 10 an agent.

In one embodiment the agent is provided to the APC in the absence of the T cell. The APC is then provided to the T cell, typically after being allowed to present the agent on its surface. The peptide may have been taken up inside the APC and presented, or simply be taken up onto the surface without entering inside the APC.

15 The duration for which the agent is contacted with the T cells will vary depending on the method used for determining recognition of the peptide. Typically 10^5 to 10^7 , preferably 5×10^5 to 10^6 PBMCs are added to each assay. In the case where agent is added directly to the assay its concentration is from 10^{-1} to $10^3 \mu\text{g/ml}$, preferably 0.5 to 50 $\mu\text{g/ml}$ or 1 to 10 $\mu\text{g/ml}$.

20 Typically the length of time for which the T cells are incubated with the agent is from 4 to 24 hours, preferably 6 to 16 hours. When using *ex vivo* PBMCs it has been found that 0.3×10^6 PBMCs can be incubated in 10 $\mu\text{g/ml}$ of peptide for 12 hours at 37°C.

25 The determination of the recognition of the agent by the T cells may be done by measuring the binding of the agent to the T cells (this can be carried out using any suitable binding assay format discussed herein). Typically T cells which bind the agent can be sorted based on this binding, for example using a FACS machine. The presence of T cells that recognise the agent will be deemed to occur if the frequency of cells sorted using the agent is above a "control" value. The frequency of antigen-30 experienced T cells is generally 1 in 10^6 to 1 in 10^3 , and therefore whether or not the sorted cells are antigen-experienced T cells can be determined.

The determination of the recognition of the agent by the T cells may be measured *in vivo*. Typically the agent is administered to the host and then a response which indicates recognition of the agent may be measured. The agent is typically administered intradermally or epidermally. The agent is typically administered by

5 contacting with the outside of the skin, and may be retained at the site with the aid of a plaster or dressing. Alternatively the agent may be administered by needle, such as by injection, but can also be administered by other methods such as ballistics (e.g. the ballistics techniques which have been used to deliver nucleic acids). EP-A-0693119 describes techniques that can typically be used to administer the agent.

10 Typically from 0.001 to 1000 µg, for example from 0.01 to 100 µg or 0.1 to 10 µg of agent is administered.

In one embodiment a product can be administered which is capable of providing the agent *in vivo*. Thus a polynucleotide capable of expressing the agent can be administered, typically in any of the ways described above for the

15 administration of the agent. The polynucleotide typically has any of the characteristics of the polynucleotide provided by the invention which is discussed below. The agent is expressed from the polynucleotide *in vivo*. Typically from 0.001 to 1000 µg, for example from 0.01 to 100 µg or 0.1 to 10 µg of polynucleotide is administered.

20 Recognition of the agent administered to the skin is typically indicated by the occurrence of inflammation (e.g. induration, erythema or oedema) at the site of administration. This is generally measured by visual examination of the site.

The method of diagnosis based on the detection of an antibody that binds the agent is typically carried out by contacting a sample from the individual (such as any

25 of the samples mentioned here, optionally processed in any manner mentioned herein) with the agent and determining whether an antibody in the sample binds the agent, such a binding indicating that the individual has, or is susceptible to coeliac disease. Any suitable format of binding assay may be used, such as any such format mentioned herein.

30

Therapy

The identification of the immunodominant epitope and other epitopes described herein allows therapeutic products to be made which target the T cells which recognise this epitope (such T cells being ones which participate in the immune response against gliadin). These findings also allow the prevention or 5 treatment of coeliac disease by suppressing (by tolerisation) an antibody or T cell response to the epitope(s).

Certain agents of the invention bind the TCR that recognises the epitope of the invention (as measured using any of the binding assays discussed above) and cause tolerisation of the T cell that carries the TCR. Such agents, optionally in 10 association with a carrier, can therefore be used to prevent or treat coeliac disease.

Generally tolerisation can be caused by the same peptides which can (after being recognised by the TCR) cause antigen specific functional activity of the T cell (such as any such activity mentioned herein, e.g. secretion of cytokines). Such agents cause tolerisation when they are presented to the immune system in a 15 'tolerising' context.

Tolerisation leads to a decrease in the recognition of a T cell or antibody epitope by the immune system. In the case of a T cell epitope this can be caused by the deletion or anergising of T cells that recognise the epitope. Thus T cell activity (for example as measured in suitable assays mentioned herein) in response to the 20 epitope is decreased. Tolerisation of an antibody response means that a decreased amount of specific antibody to the epitope is produced when the epitope is administered.

Methods of presenting antigens to the immune system in such a context are known and are described for example in Yoshida et al. Clin. Immunol. 25 Immunopathol. 82, 207-215 (1997), Thurau et al. Clin. Exp. Immunol. 109, 370-6 (1997), and Weiner et al. Res. Immunol. 148, 528-33 (1997). In particular certain routes of administration can cause tolerisation, such as oral, nasal or intraperitoneal. Tolerisation may also be accomplished via dendritic cells and tetramers presenting peptide. Particular products which cause tolerisation may be administered (e.g. in a 30 composition that also comprises the agent) to the individual. Such products include cytokines, such as cytokines that favour a Th2 response (e.g. IL-4, TGF- β or IL-10). Products or agent may be administered at a dose that causes tolerisation.

The invention provides a protein that comprises a sequence able to act as an antagonist of the T cell (which T cell recognises the agent). Such proteins and such antagonists can also be used to prevent or treat coeliac disease. The antagonist will cause a decrease in the T cell response. In one embodiment, the antagonist binds the 5 TCR of the T cell (generally in the form of a complex with HLA-DQ2 or -DQ8) but instead of causing normal functional activation causing an abnormal signal to be passed through the TCR intracellular signalling cascade, which causes the T cell to have decreased function activity (e.g. in response to recognition of an epitope, typically as measured by any suitable assay mentioned herein).

10 In one embodiment the antagonist competes with epitope to bind a component of MHC processing and presentation pathway, such as an MHC molecule (typically HLA-DQ2 or -DQ8). Thus the antagonist may bind HLA-DQ2 or -DQ8 (and thus be a peptide presented by this MHC molecule), such as peptide TP (Table 10) or a homologue thereof.

15 Methods of causing antagonism are known in the art. In one embodiment the antagonist is a homologue of the epitopes mentioned above and may have any of the sequence, binding or other properties of the agent (particularly analogues). The antagonists typically differ from any of the above epitopes (which are capable of causing a normal antigen specific function in the T cell) by 1, 2, 3, 4 or more 20 mutations (each of which may be a substitution, insertion or deletion). Such antagonists are termed "altered peptide ligands" or "APL" in the art. The mutations are typically at the amino acid positions that contact the TCR.

The antagonist may differ from the epitope by a substitution within the sequence that is equivalent to the sequence represented by amino acids 65 to 67 of A-gliadin (such antagonists are shown in Table 9). Thus preferably the antagonist has a substitution at the equivalent of position 64, 65 or 67. Preferably the substitution is 25 64W, 67W, 67M or 65T.

Since the T cell immune response to the epitope of the invention in an individual is polyclonal, more than one antagonist may need to be administered to 30 cause antagonism of T cells of the response which have different TCRs. Therefore the antagonists may be administered in a composition which comprises at least 2, 4, 6 or more different antagonists, which each antagonise different T cells.

The invention also provides a method of identifying an antagonist of a T cell (which recognises the agent), comprising contacting a candidate substance with the T cell and detecting whether the substance causes a decrease in the ability of the T cell to undergo an antigen specific response (e.g. using any suitable assay mentioned 5 herein), the detecting of any such decrease in said ability indicating that the substance is an antagonist.

In one embodiment, the antagonists (including combinations of antagonists to a particular epitope) or tolerising (T cell and antibody tolerising) agents are present in a composition comprising at least 2, 4, 6 or more antagonists or agents which 10 antagonise or tolerise to different epitopes of the invention, for example to the combinations of epitopes discussed above in relation to the agents which are a product comprising more than one substance.

Testing whether a composition is capable of causing coeliac disease

15 As mentioned above the invention provides a method of determining whether a composition is capable of causing coeliac disease comprising detecting the presence of a protein sequence which is capable of being modified by a transglutaminase to a sequence comprising the agent or epitope of the invention (such transglutaminase activity may be a human intestinal transglutaminase activity). 20 Typically this is performed by using a binding assay in which a moiety which binds to the sequence in a specific manner is contacted with the composition and the formation of sequence/moiety complex is detected and used to ascertain the presence of the agent. Such a moiety may be any suitable substance (or type of substance) mentioned herein, and is typically a specific antibody. Any suitable format of 25 binding assay can be used (such as those mentioned herein).

In one embodiment, the composition is contacted with at least 2, 5, 10 or more antibodies which are specific for epitopes of the invention from different gliadins, for example a panel of antibodies capable of recognising the combinations of epitopes discussed above in relation to agents of the invention which are a product 30 comprising more than one substance.

The composition typically comprises material from a plant that expresses a gliadin which is capable of causing coeliac disease (for example any of the gliadins

or plants mentioned herein). Such material may be a plant part, such as a harvested product (e.g. seed). The material may be processed products of the plant material (e.g. any such product mentioned herein), such as a flour or food that comprises the gliadin. The processing of food material and testing in suitable binding assays is 5 routine, for example as mentioned in Kricka LJ, *J. Biolumin. Chemilumin.* 13, 189-93 (1998).

Binding assays

10 The determination of binding between any two substances mentioned herein may be done by measuring a characteristic of either or both substances that changes upon binding, such as a spectroscopic change.

The binding assay format may be a 'band shift' system. This involves determining whether the presence of one substance (such as a candidate substance) advances or retards the progress of the other substance during gel electrophoresis.

15 The format may be a competitive binding method which determines whether the one substance is able to inhibit the binding of the other substance to an agent which is known to bind the other substance, such as a specific antibody.

Mutant gliadin proteins

20 The invention provides a gliadin protein in which an epitope sequence of the invention, or sequence which can be modified by a transglutaminase to provide such a sequence has been mutated so that it no longer causes, or is recognised by, a T cell response that recognises the epitope. In this context the term recognition refers to the TCR binding the epitope in such a way that normal (not antagonistic) antigen-specific functional activity of the T cell occurs.

Methods of identifying equivalent epitopes in other gliadins are discussed above. The wild type of the mutated gliadin is one which causes coeliac disease. Such a gliadin may have homology with SEQ ID NO:3, for example to the degree mentioned above (in relation to the analogue) across all of SEQ ID NO:3 or across 30 15, 30, 60, 100 or 200 contiguous amino acids of SEQ ID NO:3. Likewise, for other non-A-gliadins, homology will be present between the mutant and the native form of that gliadin. The sequences of other natural gliadin proteins are known in the art.

The mutated gliadin will not cause coeliac disease or will cause decreased symptoms of coeliac disease. Typically the mutation decreases the ability of the epitope to induce a T cell response. The mutated epitope may have a decreased binding to HLA-DQ2 or -DQ8, a decreased ability to be presented by an APC or a 5 decreased ability to bind to or to be recognised (i.e. cause antigen-specific functional activity) by T cells that recognise the agent. The mutated gliadin or epitope will therefore show no or reduced recognition in any of the assays mentioned herein in relation to the diagnostic aspects of the invention.

The mutation may be one or more deletions, additions or substitutions of 10 length 1 to 3, 4 to 6, 6 to 10, 11 to 15 or more in the epitope, for example across sequence SEQ ID NO:2 or across any of SEQ ID NOS: 18-22, 31-36, 39-44, and 46; or across equivalents thereof. Preferably the mutant gliadin has at least one mutation in the sequence SEQ ID NO:1. A preferred mutation is at position 65 in A-gliadin (or in an equivalent position in other gliadins). Typically the naturally occurring 15 glutamine at this position is substituted to any of the amino acids shown in Table 3, preferably to histidine, tyrosine, tryptophan, lysine, proline, or arginine.

The invention thus also provides use of a mutation (such any of the mutations in any of the sequences discussed herein) in an epitope of a gliadin protein, which epitope is an epitope of the invention, to decrease the ability of the gliadin protein to 20 cause coeliac disease.

In one embodiment the mutated sequence is able to act as an antagonist. Thus the invention provides a protein that comprises a sequence which is able to bind 25 to a T cell receptor, which T cell receptor recognises an agent of the invention, and which sequence is able to cause antagonism of a T cell that carries such a T cell receptor.

The invention also provides proteins which are fragments of the above mutant gliadin proteins, which are at least 15 amino acids long (e.g. at least 30, 60, 100, 150, 200, or 250 amino acids long) and which comprise the mutations discussed above which decrease the ability of the gliadin to be recognised. Any of the mutant 30 proteins (including fragments) mentioned herein may also be present in the form of fusion proteins, for example with other gliadins or with non-gliadin proteins.

The equivalent wild type protein to the mutated gliadin protein is typically from a graminaceous monocotyledon, such as a plant of genus *Triticum*, e.g. wheat, rye, barley, oats or triticale. The protein is typically an α , $\alpha\beta$, β , γ or ω gliadin. The gliadin may be an A-gliadin.

5

Kits

The invention also provides a kit for carrying out the method comprising one or more agents and optionally a means to detect the recognition of the agent by the T cell. Typically the different agents are provided for simultaneous, separate or

10 sequential use. Typically the means to detect recognition allows or aids detection based on the techniques discussed above.

Thus the means may allow detection of a substance secreted by the T cells after recognition. The kit may thus additionally include a specific binding moiety for the substance, such as an antibody. The moiety is typically specific for IFN- γ . The 15 moiety is typically immobilised on a solid support. This means that after binding the moiety the substance will remain in the vicinity of the T cell which secreted it. Thus "spots" of substance/moiety complex are formed on the support, each spot representing a T cell which is secreting the substance. Quantifying the spots, and typically comparing against a control, allows determination of recognition of the 20 agent.

The kit may also comprise a means to detect the substance/moiety complex. A detectable change may occur in the moiety itself after binding the substance, such as a colour change. Alternatively a second moiety directly or indirectly labelled for detection may be allowed to bind the substance/moiety complex to allow the 25 determination of the spots. As discussed above the second moiety may be specific for the substance, but binds a different site on the substance than the first moiety.

The immobilised support may be a plate with wells, such as a microtitre plate. Each assay can therefore be carried out in a separate well in the plate.

The kit may additionally comprise medium for the T cells, detection moieties 30 or washing buffers to be used in the detection steps. The kit may additionally comprise reagents suitable for the separation from the sample, such as the separation of PBMCs or T cells from the sample. The kit may be designed to allow detection of

the T cells directly in the sample without requiring any separation of the components of the sample.

The kit may comprise an instrument which allows administration of the agent, such as intradermal or epidermal administration. Typically such an instrument 5 comprises plaster, dressing or one or more needles. The instrument may allow ballistic delivery of the agent. The agent in the kit may be in the form of a pharmaceutical composition.

The kit may also comprise controls, such as positive or negative controls.

The positive control may allow the detection system to be tested. Thus the positive 10 control typically mimics recognition of the agent in any of the above methods.

Typically in the kits designed to determine recognition *in vitro* the positive control is a cytokine. In the kit designed to detect *in vivo* recognition of the agent the positive control may be antigen to which most individuals should respond.

The kit may also comprise a means to take a sample containing T cells from 15 the host, such as a blood sample. The kit may comprise a means to separate mononuclear cells or T cells from a sample from the host.

Polynucleotides, cells, transgenic mammals and antibodies

The invention also provides a polynucleotide which is capable of expression 20 to provide the agent or mutant gliadin proteins. Typically the polynucleotide is DNA or RNA, and is single or double stranded. The polynucleotide will preferably comprise at least 50 bases or base pairs, for example 50 to 100, 100 to 500, 500 to 1000 or 1000 to 2000 or more bases or base pairs. The polynucleotide therefore 25 comprises a sequence which encodes the sequence of SEQ ID NO: 1 or 2 or any of the other agents mentioned herein. To the 5' and 3' of this coding sequence the polynucleotide of the invention has sequence or codons which are different from the sequence or codons 5' and 3' to these sequences in the corresponding gliadin gene.

5' and/or 3' to the sequence encoding the peptide the polynucleotide has 30 coding or non-coding sequence. Sequence 5' and/or 3' to the coding sequence may comprise sequences which aid expression, such as transcription and/or translation, of the sequence encoding the agent. The polynucleotide may be capable of expressing the agent prokaryotic or eukaryotic cell. In one embodiment the polynucleotide is

capable of expressing the agent in a mammalian cell, such as a human, primate or rodent (e.g. mouse or rat) cell.

A polynucleotide of the invention may hybridise selectively to a polynucleotide that encodes SEQ ID NO:3 at a level significantly above background.

5. Selective hybridisation is typically achieved using conditions of medium to high stringency (for example 0.03M sodium chloride and 0.03M sodium citrate at from about 50°C to about 60°C). However, such hybridisation may be carried out under any suitable conditions known in the art (see Sambrook *et al* (1989), *Molecular Cloning: A Laboratory Manual*). For example, if high stringency is required, suitable 10 conditions include 0.2 x SSC at 60°C. If lower stringency is required, suitable conditions include 2 x SSC at 60°C.

Agents or proteins of the invention may be encoded by the polynucleotides described herein.

The polynucleotide may form or be incorporated into a replicable vector.

15. Such a vector is able to replicate in a suitable cell. The vector may be an expression vector. In such a vector the polynucleotide of the invention is operably linked to a control sequence which is capable of providing for the expression of the polynucleotide. The vector may contain a selectable marker, such as the ampicillin resistance gene.

20. The polynucleotide or vector may be present in a cell. Such a cell may have been transformed by the polynucleotide or vector. The cell may express the agent. The cell will be chosen to be compatible with the said vector and may for example be a prokaryotic (bacterial), yeast, insect or mammalian cell. The polynucleotide or vector may be introduced into host cells using conventional techniques including 25 calcium phosphate precipitation, DEAE-dextran transfection, or electroporation.

30. The invention provides processes for the production of the proteins of the invention by recombinant means. This may comprise (a) cultivating a transformed cell as defined above under conditions that allow the expression of the protein; and preferably (b) recovering the expressed polypeptide. Optionally, the polypeptide may be isolated and/or purified, by techniques known in the art.

The invention also provides TCRs which recognise (or bind) the agent, or fragments thereof which are capable of such recognition (or binding). These can be

present in the any form mentioned herein (e.g. purity) discussed herein in relation to the protein of the invention. The invention also provides T cells which express such TCRs which can be present in any form (e.g. purity) discussed herein for the cells of the invention.

5 The invention also provides monoclonal or polyclonal antibodies which specifically recognise the agents (such as any of the epitopes of the invention) and which recognise the mutant gliadin proteins (and typically which do not recognise the equivalent wild-type gliadins) of the invention, and methods of making such antibodies. Antibodies of the invention bind specifically to these substances of the
10 invention:

For the purposes of this invention, the term "antibody" includes antibody fragments such as Fv, F(ab) and F(ab)₂ fragments, as well as single-chain antibodies.

A method for producing a polyclonal antibody comprises immunising a suitable host animal, for example an experimental animal, with the immunogen and
15 isolating immunoglobulins from the serum. The animal may therefore be inoculated with the immunogen, blood subsequently removed from the animal and the IgG fraction purified. A method for producing a monoclonal antibody comprises immortalising cells which produce the desired antibody. Hybridoma cells may be produced by fusing spleen cells from an inoculated experimental animal with tumour
20 cells (Kohler and Milstein (1975) *Nature* 256, 495-497).

An immortalized cell producing the desired antibody may be selected by a conventional procedure. The hybridomas may be grown in culture or injected intraperitoneally for formation of ascites fluid or into the blood stream of an allogenic host or immunocompromised host. Human antibody may be prepared by *in*
25 *vitro* immunisation of human lymphocytes, followed by transformation of the lymphocytes with Epstein-Barr virus.

For the production of both monoclonal and polyclonal antibodies, the experimental animal is suitably a goat, rabbit, rat or mouse. If desired, the immunogen may be administered as a conjugate in which the immunogen is coupled,
30 for example via a side chain of one of the amino acid residues, to a suitable carrier. The carrier molecule is typically a physiologically acceptable carrier. The antibody obtained may be isolated and, if desired, purified.

The polynucleotide, agent, protein or antibody of the invention, may carry a detectable label. Detectable labels which allow detection of the secreted substance by visual inspection, optionally with the aid of an optical magnifying means, are preferred. Such a system is typically based on an enzyme label which causes colour 5 change in a substrate, for example alkaline phosphatase causing a colour change in a substrate. Such substrates are commercially available, e.g. from BioRad. Other suitable labels include other enzymes such as peroxidase, or protein labels, such as biotin; or radioisotopes, such as ^{32}P or ^{35}S . The above labels may be detected using known techniques.

10 Polynucleotides, agents, proteins, antibodies or cells of the invention may be in substantially purified form. They may be in substantially isolated form, in which case they will generally comprise at least 80% e.g. at least 90, 95, 97 or 99% of the polynucleotide, peptide, antibody, cells or dry mass in the preparation. The polynucleotide, agent, protein or antibody is typically substantially free of other 15 cellular components. The polynucleotide, agent, protein or antibody may be used in such a substantially isolated, purified or free form in the method or be present in such forms in the kit.

The invention also provides a transgenic non-human mammal which 20 expresses a TCR of the invention. This may be any of the mammals discussed herein (e.g. in relation to the production of the antibody). Preferably the mammal has, or is susceptible, to coeliac disease. The mammal may also express HLA-DQ2 or -DQ8 or HLA-DR3-DQ2 and/or may be given a diet comprising a gliadin which cause coeliac disease (e.g. any of the gliadin proteins mentioned herein). Thus the mammal may act as an animal model for coeliac disease.

25 The invention also provides a method of identifying a product which is therapeutic for coeliac disease comprising administering a candidate substance to a mammal of the invention which has, or which is susceptible to, coeliac disease and determining whether substance prevents or treats coeliac disease in the mammal, the prevention or treatment of coeliac disease indicating that the substance is a 30 therapeutic product. Such a product may be used to treat or prevent coeliac disease.

The invention provides therapeutic (including prophylactic) agents or diagnostic substances (the agents, proteins and polynucleotides of the invention).

These substances are formulated for clinical administration by mixing them with a pharmaceutically acceptable carrier or diluent. For example they can be formulated for topical, parenteral, intravenous, intramuscular, subcutaneous, intraocular, 5 intradermal, epidermal or transdermal administration. The substances may be mixed with any vehicle which is pharmaceutically acceptable and appropriate for the desired route of administration. The pharmaceutically carrier or diluent for injection may be, for example, a sterile or isotonic solution such as Water for Injection or physiological saline, or a carrier particle for ballistic delivery.

The dose of the substances may be adjusted according to various parameters, 10 especially according to the agent used; the age, weight and condition of the patient to be treated; the mode of administration used; the severity of the condition to be treated; and the required clinical regimen. As a guide, the amount of substance administered by injection is suitably from 0.01 mg/kg to 30 mg/kg, preferably from 0.1 mg/kg to 10 mg/kg.

15 The routes of administration and dosages described are intended only as a guide since a skilled practitioner will be able to determine readily the optimum route of administration and dosage for any particular patient and condition.

The substances of the invention may thus be used in a method of treatment of the human or animal body, or in a diagnostic method practised on the human body. 20 In particular they may be used in a method of treating or preventing coeliac disease. The invention also provide the agents for use in a method of manufacture of a medicament for treating or preventing coeliac disease. Thus the invention provides a method of preventing or treating coeliac disease comprising administering to a human in need thereof a substance of the invention (typically a non-toxic effective 25 amount thereof).

The agent of the invention can be made using standard synthetic chemistry techniques, such as by use of an automated synthesizer. The agent may be made from a longer polypeptide e.g. a fusion protein, which polypeptide typically comprises the sequence of the peptide. The peptide may be derived from the 30 polypeptide by for example hydrolysing the polypeptide, such as using a protease; or by physically breaking the polypeptide. The polynucleotide of the invention can be made using standard techniques, such as by using a synthesiser.

Plant cells and plants that express mutant gliadin proteins or express proteins comprising sequences which can act as antagonists

The cell of the invention may be a plant cell, such as a cell of a graminaceous 5 monocotyledonous species. The species may be one whose wild-type form expresses gliadins, such as any of the gliadin proteins mentioned herein (including gliadins with any degree of homology to SEQ ID NO:3 mentioned herein). Such a gliadin may cause coeliac disease in humans. The cell may be of wheat, maize, oats, rye, rice, barley, triticale, sorghum, or sugar cane. Typically the cell is of the *Triticum* 10 genus, such as *aestivum*, *spelta*, *polonicum* or *monococcum*.

The plant cell of the invention is typically one which does not express a wild-type gliadin (such as any of the gliadins mentioned herein which may cause coeliac disease), or one which does not express a gliadin comprising a sequence that can be 15 recognised by a T cell that recognises the agent. Thus if the wild-type plant cell did express such a gliadin then it may be engineered to prevent or reduce the expression of such a gliadin or to change the amino acid sequence of the gliadin so that it no longer causes coeliac disease (typically by no longer expressing the epitope of the invention).

This can be done for example by introducing mutations into 1, 2, 3 or more or 20 all of such gliadin genes in the cell, for example into coding or non-coding (e.g. promoter regions). Such mutations can be any of the type or length of mutations discussed herein (e.g., in relation to homologous proteins). The mutations can be introduced in a directed manner (e.g., using site directed mutagenesis or homologous recombination techniques) or in a random manner (e.g. using a mutagen, and then 25 typically selecting for mutagenised cells which no longer express the gliadin (or a gliadin sequence which causes coeliac disease)).

In the case of plants or plant cells that express a protein that comprises a sequence able to act as an antagonist such a plant or plant cell may express a wild-type gliadin protein (e.g. one which causes coeliac disease). Preferably though the 30 presence of the antagonist sequence will cause reduced coeliac disease symptoms (such as no symptoms) in an individual who ingests a food comprising protein from the plant or plant cell.

The polynucleotide which is present in (or which was transformed into) the plant cell will generally comprise promoter capable of expressing the mutant gliadin protein in the plant cell. Depending on the pattern of expression desired, the promoter may be constitutive, tissue- or stage-specific; and/or inducible. For example, strong 5 constitutive expression in plants can be obtained with the CAMV 35S, Rubisco ssu, or histone promoters. Also, tissue-specific or stage-specific promoters may be used to target expression of protein of the invention to particular tissues in a transgenic plant or to particular stages in its development. Thus, for example seed-specific, root-specific, leaf-specific, flower-specific etc promoters may be used. Seed-specific 10 promoters include those described by Dalta *et al* (Biotechnology Ann. Rev. (1997), 3, pp.269-296). Particular examples of seed-specific promoters are napin promoters (EP-A-0 255, 378), phaseolin promoters, glutenine promoters, helianthenine promoters (WO92/17580), albumin promoters (WO98/45460), oleosin promoters (WO98/45461) and ATS1 and ATS3 promoters (PCT/US98/06798).

15 The cell may be in any form. For example, it may be an isolated cell, e.g. a protoplast, or it may be part of a plant tissue, e.g. a callus, or a tissue excised from a plant, or it may be part of a whole plant. The cell may be of any type (e.g. of any type of plant part). For example, an undifferentiated cell, such as a callus cell; or a differentiated cell, such as a cell of a type found in embryos, pollen, roots, shoots or 20 leaves. Plant parts include roots; shoots; leaves; and parts involved in reproduction, such as pollen, ova, stamens, anthers, petals, sepals and other flower parts.

The invention provides a method of obtaining a transgenic plant cell comprising transforming a plant cell with a polynucleotide or vector of the invention to give a transgenic plant cell. Any suitable transformation method may be used (in 25 the case of wheat the techniques disclosed in Vasil V *et al*, Biotechnology 10, 667-674 (1992) may be used). Preferred transformation techniques include electroporation of plant protoplasts and particle bombardment. Transformation may thus give rise to a chimeric tissue or plant in which some cells are transgenic and some are not.

30 The cell of the invention or thus obtained cell may be regenerated into a transgenic plant by techniques known in the art. These may involve the use of plant growth substances such as auxins, gibberellins and/or cytokinins to stimulate the

growth and/or division of the transgenic cell. Similarly, techniques such as somatic embryogenesis and meristem culture may be used. Regeneration techniques are well known in the art and examples can be found in, e.g. US 4,459,355, US 4,536,475, US 5,464,763, US 5,177,010, US 5,187,073, EP 267,159, EP 604, 662, EP 672, 752, 5 US 4,945,050, US 5,036,006, US 5,100,792, US 5,371,014, US 5,478,744, US 5,179,022, US 5,565,346, US 5,484,956, US 5,508,468, US 5,538,877, US 5,554,798, US 5,489,520, US 5,510,318, US 5,204,253, US 5,405,765, EP 442,174, EP 486,233, EP 486,234, EP 539,563, EP 674,725, WO91/02071 and WO 95/06128.

10 In many such techniques, one step is the formation of a callus, i.e. a plant tissue comprising expanding and/or dividing cells. Such calli are a further aspect of the invention as are other types of plant cell cultures and plant parts. Thus, for example, the invention provides transgenic plant tissues and parts, including embryos, meristems, seeds, shoots, roots, stems, leaves and flower parts. These may be chimeric in the sense that some of their cells are cells of the invention and some 15 are not. Transgenic plant parts and tissues, plants and seeds of the invention may be of any of the plant species mentioned herein.

Regeneration procedures will typically involve the selection of transformed cells by means of marker genes.

20 The regeneration step gives rise to a first generation transgenic plant. The invention also provides methods of obtaining transgenic plants of further generations from this first generation plant. These are known as progeny transgenic plants. Progeny plants of second, third, fourth, fifth, sixth and further generations may be obtained from the first generation transgenic plant by any means known in the art.

25 Thus, the invention provides a method of obtaining a transgenic progeny plant comprising obtaining a second-generation transgenic progeny plant from a first-generation transgenic plant of the invention, and optionally obtaining transgenic plants of one or more further generations from the second-generation progeny plant thus obtained.

30 Progeny plants may be produced from their predecessors of earlier generations by any known technique. In particular, progeny plants may be produced by:

obtaining a transgenic seed from a transgenic plant of the invention belonging to a previous generation, then obtaining a transgenic progeny plant of the invention belonging to a new generation by growing up the transgenic seed; and/or

propagating clonally a transgenic plant of the invention belonging to a

5 previous generation to give a transgenic progeny plant of the invention belonging to a new generation; and/or

crossing a first-generation transgenic plant of the invention belonging to a previous generation with another compatible plant to give a transgenic progeny plant of the invention belonging to a new generation; and optionally

10 obtaining transgenic progeny plants of one or more further generations from the progeny plant thus obtained.

These techniques may be used in any combination. For example, clonal propagation and sexual propagation may be used at different points in a process that gives rise to a transgenic plant suitable for cultivation. In particular, repetitive back-crossing with a plant taxon with agronomically desirable characteristics may be undertaken. Further steps of removing cells from a plant and regenerating new plants therefrom may also be carried out.

Also, further desirable characteristics may be introduced by transforming the cells, plant tissues, plants or seeds, at any suitable stage in the above process, to

20 introduce desirable coding sequences other than the polynucleotides of the invention. This may be carried out by the techniques described herein for the introduction of polynucleotides of the invention.

For example, further transgenes may be selected from those coding for other herbicide resistance traits, e.g. tolerance to: Glyphosate (e.g. using an EPSP synthase gene (e.g. EP-A-0 293,358) or a glyphosate oxidoreductase (WO 92/000377) gene); or tolerance to fosametin; a dihalobenzonitrile; glufosinate, e.g. using a phosphinothrycin acetyl transferase (PAT) or glutamine synthase gene (cf. EP-A-0 242,236); asulam, e.g. using a dihydropteroate synthase gene (EP-A-0 369,367); or a sulphonylurea, e.g. using an ALS gene); diphenyl ethers such as acifluorfen or

30 oxyfluorfen, e.g. using a protoporphyrin oxidase gene); an oxadiazole such as oxadiazon; a cyclic imide such as chlorophthalim; a phenyl pyrazole such as TNP, or a phenoplylate or carbamate analogue thereof.

Similarly, genes for beneficial properties other than herbicide tolerance may be introduced. For example, genes for insect resistance may be introduced, notably genes encoding *Bacillus thuringiensis* (*Bt*) toxins. Likewise, genes for disease resistance may be introduced, e.g. as in WO91/02701 or WO95/06128.

5 Typically, a protein of the invention is expressed in a plant of the invention. Depending on the promoter used, this expression may be constitutive or inducible. Similarly, it may be tissue- or stage-specific, i.e. directed towards a particular plant tissue (such as any of the tissues mentioned herein) or stage in plant development.

10 The invention also provides methods of obtaining crop products by harvesting, and optionally processing further, transgenic plants of the invention. By crop product is meant any useful product obtainable from a crop plant.

15 **Products that contain mutant gliadin proteins or proteins that comprise sequence capable of acting as an antagonist**

20 The invention provides a product that comprises the mutant gliadin proteins or protein that comprises sequence capable of acting as an antagonist. This is typically derived from or comprise plant parts from plants mentioned herein which express such proteins. Such a product may be obtainable directly by harvesting or indirectly, by harvesting and further processing the plant of the invention. Directly obtainable products include grains. Alternatively, such a product may be obtainable indirectly, by harvesting and further processing. Examples of products obtainable by further processing are flour or distilled alcoholic beverages; food products made from directly obtained or further processed material, e.g. baked products (e.g. bread) made from flour. Typically such food products, which are ingestible and digestible (i.e. non-toxic and of nutrient value) by human individuals.

25 In the case of food products that comprise the protein which comprises an antagonist sequence the food product may also comprise wild-type gliadin, but preferably the antagonist is able to cause a reduction (e.g. completely) in the coeliac disease symptoms after such food is ingested.

30 The invention is illustrated by the following nonlimiting Examples:

Example 1

We carried out epitope mapping in Coeliac disease by using a set of 51 synthetic 15-mer peptides that span the complete sequence of a fully characterized a-gliadin, "A-gliadin" (see Table 1). A-Gliadin peptides were also individually treated with tTG to generate products that might mimic those produced *in vivo*³. We also 5 sought to study Coeliac disease patients at the point of initiation of disease relapse to avoid the possibility that epitope "spreading" or "exhaustion" may have occurred, as described in experimental infectious and autoimmune diseases.

Clinical and A-gliadin specific T-cell responses with 3 and 10 day bread challenge

10 In a pilot study, two subjects with Coeliac disease in remission, defined by absence of serum anti-endomysial antibody (EMA), on a gluten free diet were fed four slices of standard gluten-containing white bread daily in addition to their usual gluten free diet. Subject 1 ceased bread because of abdominal pain, mouth ulcers and mild diarrhoea after three days, but Subject 2 continued for 10 days with only 15 mild nausea at one week. The EMA became positive in Subject 2 one week after the bread challenge, indicating the bread used had caused a relapse of Coeliac disease. But in Subject 1, EMA remained negative up to two months after bread challenge. In both subjects, symptoms that appeared with bread challenge resolved within two days after returning to gluten free diet.

20 PBMC responses in IFN γ ELISPOT assays to A-gliadin peptides were not found before or during bread challenge. But from the day after bread withdrawal (Day 4) in Subject 1 a single pool of 5 overlapping peptides spanning A-gliadin 51-85 (Pool 3) treated with tTG showed potent IFN γ responses (see Figure 1a). In Subject 1, the PBMC IFN γ response to A-gliadin peptide remained targeted to Pool 3 25 alone and was maximal on Day 8. The dynamics and magnitude of the response to Pool 3 was similar to that elicited by α -chymotrypsin digested gliadin. PBMC IFN γ responses to tTG-treated Pool 3 were consistently 5 to 12-fold greater than Pool 3 not treated with tTG, and responses to α -chymotrypsin digested gliadin were 3 to 10-fold greater if treated with tTG. In Subject 2, Pool 3 treated with tTG was also the only 30 immunogenic set of A-gliadin peptides on Day 8, but this response was weaker than Subject 1, was not seen on Day 4 and by Day 11 the response to Pool 3 had diminished and other tTG-treated pools of A-gliadin peptides elicited stronger IFN α

responses (see Figure 1b).

The pilot study indicated that the initial T cell response in these Coeliac disease subjects was against a single tTG-treated A-gliadin pool of five peptides and was readily measured in peripheral blood. But if antigen exposure is continued for 5 ten days instead of three, T cell responses to other A-gliadin peptides appear, consistent with epitope spreading.

Coeliac disease-specific IFN- γ induction by tTG-treated A-gliadin peptides

In five out of six further Coeliac disease subjects on gluten free diet (see 10 Table 1), bread challenge for three days identified tTG-treated peptides in Pool 3, and in particular, peptides corresponding to 56-70 (12) and 60-75 (13) as the sole A-gliadin components eliciting IFN γ from PBMC (see Figure 2). IL-10 ELISPOT assays run in parallel to IFN γ ELISPOT showed no IL-10 response to tTG-treated peptides 12 or 13. In one subject, there were no IFN γ responses to any A-gliadin 15 peptide or α -chymotrypsin digested gliadin before, during or up to four days after bread challenge. In none of these Coeliac disease subjects did EMA status change from baseline when measured for up to two months after bread challenge.

PBMC from four healthy, EMA-negative subjects with the HLA-DQ alleles $\alpha 1^*0501$, $\beta 1^*0201$ (ages 28-52, 2 females) who had been challenged for three days 20 with bread after following a gluten free diet for one month, showed no IFN γ responses above the negative control to any of the A-gliadin peptides with or without tTG treatment. Thus, induction of IFN γ in PBMC to tTG-treated Pool 3 and A-gliadin peptides 56-70 (12) and 60-75 (13) were Coeliac disease specific (7/8 vs. 0/4, p<0.01 by Chi-squared analysis).

25

Fine mapping of the minimal A-gliadin T cell epitope

tTG-treated peptides representing truncations of A-gliadin 56-75 revealed that the same core peptide sequence QPQLP (SEQ ID NO:9) was essential for antigenicity in all of the five Coeliac disease subjects assessed (see Figure 3). PBMC 30 IFN γ responses to tTG-treated peptides spanning this core sequence beginning with the 7-mer PQPQLPY (SEQ ID NO:4) and increasing in length, indicated that the tTG-treated 17-mer QLQPFPQPQLPYPQPQS (SEQ ID NO:10) (A-gliadin 57-73)

possessed optimal activity in the $\text{IFN}\gamma$ ELISPOT (see Figure 4).

Deamidation of Q65 by tTG generates the immunodominant T cell epitope in A-gliadin

5 HPLC analysis demonstrated that tTG treatment of A-gliadin 56-75 generated a single product that eluted marginally later than the parent peptide. Amino acid sequencing indicated that out of the six glutamine (Q) residues contained in A-gliadin 56-75, Q65 was preferentially deamidated by tTG (see Figure 5). Bioactivity of peptides corresponding to serial expansions from the core A-gliadin 62-68
10 sequence in which glutamate (E) replaced Q65, was equivalent to the same peptides with Q65 after tTG-treatment (see Figure 4a). Replacement of Q57 and Q72 by E together or alone, with E65 did not enhance antigenicity of the 17-mer in the three Coeliac disease subjects studied (see Figure 6). Q57 and Q72 were investigated because glutamine residues followed by proline in gliadin peptides are not
15 deamidated by tTG in vitro (W. Vader et al, Proceedings 8th International Symposium Coeliac Disease). Therefore, the immunodominant T cell epitope was defined as QLQPFPQPEELPYPQPQS (SEQ ID NO:2).

Immunodominant T cell epitope response is DQ2-restricted and CD4 dependent

20 In two Coeliac disease subjects homozygous for HLA-DQ $\alpha 1^*0501$, $\beta 1^*0201$, anti-DQ monoclonal antibody blocked the ELISPOT $\text{IFN}\gamma$ response to tTG-treated A-gliadin 56-75, but anti-DP and -DR antibody did not (see Figure 7). Anti-CD4 and anti-CD8 magnetic bead depletion of PBMC from two Coeliac disease subjects indicated the $\text{IFN}\gamma$ response to tTG-treated A-gliadin 56-75 is CD4 T cell-mediated.
25

Discussion

In this study we describe a rather simple dietary antigen challenge using standard white bread to elicit a transient population of CD4 T cells in peripheral
30 blood of Coeliac disease subjects responsive to a tTG-treated A-gliadin 17-mer with the sequence: QLQPFPQPEELPYPQPQS (SEQ ID NO:2) (residues 57-73). The immune response to A-gliadin 56-75 (Q→E65) is restricted to the Coeliac disease-

associated HLA allele, DQ $\alpha 1^*0501$, $\beta 1^*0201$. Tissue transglutaminase action in vitro selectively deamidates Q65. Elicited peripheral blood IFNg responses to synthetic A-gliadin peptides with the substitution Q→E65 is equivalent to tTG-treated Q65 A-gliadin peptides; both stimulate up to 10-fold more T cells in the IFNg 5 ELISPOT than unmodified Q65 A-gliadin peptides.

We have deliberately defined this Coeliac disease-specific T cell epitope using in vivo antigen challenge and short-term ex vivo immune assays to avoid the possibility of methodological artifacts that may occur with the use of T cell clones in epitope mapping. Our findings indicate that peripheral blood T cell responses to 10 ingestion of gluten are rapid but short-lived and can be utilized for epitope mapping. In vivo antigen challenge has also shown there is a temporal hierarchy of immune responses to A-gliadin peptides; A-gliadin 57-73 modified by tTG not only elicits the strongest IFNg response in PBMC but it is also the first IFNg response to appear.

Because we have assessed only peptides spanning A-gliadin, there may be 15 other epitopes in other gliadins of equal or greater importance in the pathogenesis of Coeliac disease. Indeed, the peptide sequence at the core of the epitope in A-gliadin that we have identified PQPQLPY (SEQ ID NO:4) is shared by several other gliadins (SwissProt and Trembl accession numbers: P02863, Q41528, Q41531, Q41533, Q9ZP09, P04722, P04724, P18573). However, A-gliadin peptides that have 20 previously been shown to possess bioactivity in biopsy challenge and in vivo studies (for example: 31-43, 44-55, and 206-217)^{4,5} did not elicit IFNg responses in PBMC following three day bread challenge in Coeliac disease subjects. These peptides may be "secondary" T cell epitopes that arise with spreading of the immune response.

25 **Example 2**

The effect on T cell recognition of substitutions in the immunodominant epitope

The effect of substituting the glutamate at position 65 in the 57-73 A-gliadin epitope was determined by measuring peripheral blood responses against the substituted epitopes in an IFNg ELISPOT assay using synthetic peptides (at 50 30 μ g/ml). The responses were measured in 3 Coeliac disease subjects 6 days after commencing gluten challenge (4 slices bread daily for 3 days). Results are shown in table 3 and Figure 8. As can be seen substitution of the glutamate to histidine,

tyrosine, tryptophan, lysine, proline or arginine stimulated a response whose magnitude was less than 10% of the magnitude of the response to the immunodominant epitope. Thus mutation of A-gliadin at this position could be used to produce a mutant gliadin with reduced or absent immunoreactivity.

5

Example 3

Testing the immunoreactivity of equivalent peptides from other naturally occurring gliadins

The immunoreactivity of equivalent peptides from other naturally occurring wheat gliadins was assessed using synthetic peptides corresponding to the naturally occurring sequences which were then treated with transglutaminase. These peptides were tested in an ELISPOT in the same manner and with PBMCs from the same subjects as described in Example 2. At least five of the peptides show immunoreactivity comparable to the A-gliadin 57-73 E65 peptide (after transglutaminase treatment) indicating that other gliadin proteins in wheat are also likely to induce this Coeliac disease-specific immune response (Table 4 and Figure 9).

Methods

20 *Subjects:* Patients used in the study attended a Coeliac Clinic in Oxford, United Kingdom. Coeliac disease was diagnosed on the basis of typical small intestinal histology, and normalization of symptoms and small intestinal histology with gluten free diet.

25 *Tissue typing:* Tissue typing was performed using DNA extracted from EDTA-anticoagulated peripheral blood. HLA-DQA and DQB genotyping was performed by PCR using sequence-specific primer mixes⁶⁸.

30 *Anti-endomysial antibody assay:* EMA were detected by indirect immunofluorescence using patient serum diluted 1:5 with monkey oesophagus, followed by FITC-conjugated goat anti-human IgA. IgA was quantitated prior to EMA, none of the subjects were IgA deficient.

Antigen Challenge: Coeliac disease subjects following a gluten free diet, consumed 4 slices of gluten-containing bread (50g/slice, Sainsbury's "standard white sandwich bread") daily for 3 or 10 days. EMA was assessed the week before and up to two months after commencing the bread challenge. Healthy subjects who had followed a gluten free diet for four weeks, consumed their usual diet including four slices of gluten-containing bread for three days, then returned to gluten free diet for a further six days.

10 *IFN γ and IL-10 ELISPOT:* PBMC were prepared from 50-100 ml of venous blood by Ficoll-Hypaque density centrifugation. After three washes, PBMC were resuspended in complete RPMI containing 10% heat inactivated human AB serum. ELISPOT assays for single cell secretion of IFN γ and IL-10 were performed using commercial kits (Mabtech; Stockholm, Sweden) with 96-well plates (MAIP-S-45; Millipore, Bedford, MA) according to the manufacturers instructions (as described elsewhere⁹) with 2-5x10⁵ (IFN γ) or 0.4-1x10⁵ (IL-10) PBMC in each well. Peptides were assessed in duplicate wells, and *Mycobacterium tuberculosis* purified protein derivative (PPD RT49) (Serum Institute; Copenhagen, Denmark) (20 μ g/ml) was included as a positive control in all assays.

20 *Peptides:* Synthetic peptides were purchased from Research Genetics (Huntsville, Alabama) Mass-spectroscopy and HPLC verified peptides' authenticity and >70% purity. Digestion of gliadin (Sigma; G-3375) (100 mg/ml) with α -chymotrypsin (Sigma; C-3142) 200:1 (w/w) was performed at room temperature in 0.1 M NH₄HCO₃ with 2M urea and was halted after 24 h by heating to 98°C for 10 minutes. After centrifugation (13,000g, 10 minutes), the gliadin digest supernatant was filter-sterilized (0.2 mm). Digestion of gliadin was verified by SDS-PAGE and protein concentration assessed. α -Chymotrypsin-digested gliadin (640 μ g/ml) and synthetic gliadin peptides (15-mers: 160 μ g/ml, other peptides: 0.1 mM) were individually treated with tTG (Sigma; T-5398) (50 μ g/ml) in PBS + CaCl₂ 1 mM for 2 h at 37°C. Peptides and peptide pools were aliquotted into sterile 96-well plates and stored frozen at -20°C until use.

Amino acid sequencing of peptides: Reverse phase HPLC was used to purify the peptide resulting from tTG treatment of A-gliadin 56-75. A single product was identified and subjected to amino acid sequencing (automated sequencer Model 5 494A, Applied Biosystems, Foster City, California). The sequence of unmodified G56-75 was confirmed as: LQLQPFPQPQLPYPQPQSFP (SEQ ID NO:5), and tTG treated G56-75 was identified as: LQLQPFPQPELPYPQPQSFP (SEQ ID NO:11). Deamidation of glutamyl residues was defined as the amount (pmol) of glutamate recovered expressed as a percent of the combined amount of glutamine and 10 glutamate recovered in cycles 2, 4, 8, 10, 15 and 17 of the amino acid sequencing. Deamidation attributable to tTG was defined as (% deamidation of glutamine in the tTG treated peptide - % deamidation in the untreated peptide) / (100 - % deamidation in the untreated peptide).

CD4/CD8 and HLA Class II Restriction: Anti-CD4 or anti-CD8 coated magnetic 15 beads (Dynal, Oslo, Norway) were washed four times with RPMI then incubated with PBMC in complete RPMI containing 10% heat inactivated human AB serum (5×10^6 cells/ml) for 30 minutes on ice. Beads were removed using a magnet and cells remaining counted. In vivo HLA-class II restriction of the immune response to tTG-treated A-gliadin 56-75 was established by incubating PBMC (5×10^6 cells/ml) 20 with anti-HLA-DR (L243), -DQ (L2), and -DP (B7.21) monoclonal antibodies (10 μ g/ml) at room temperature for one hour prior to the addition of peptide.

Example 4

Mucosal integrin expression by gliadin -specific peripheral blood lymphocytes

25 Interaction between endothelial and lymphocyte addressins facilitates homing of organ-specific lymphocytes. Many addressins are known. The heterodimer $\alpha_4\beta_7$ is specific for lamina propria gut and other mucosal lymphocytes, and $\alpha^E\beta_7$ is specific and intra-epithelial lymphocytes in the gut and skin. Approximately 30% of peripheral blood CD4 T cells express $\alpha_4\beta_7$ and are presumed to be in transit to a 30 mucosal site, while 5% of peripheral blood T cells express $\alpha^E\beta_7$. Immunomagnetic beads coated with antibody specific for α^E or β_7 , deplete PBMC of cells expressing $\alpha^E\beta_7$ or $\alpha^E\beta_7$ and $\alpha_4\beta_7$, respectively. In combination with ELISpot assay,

immunomagnetic bead depletion allows determination of gliadin-specific T cell addressin expression that may identify these cells as homing to a mucosal surface. Interestingly, gluten challenge *in vivo* is associated with rapid influx of CD4 T cells to the small intestinal lamina propria (not intra-epithelial sites), where over 90% 5 lymphocytes express $\alpha_4\beta_7$.

Immunomagnetic beads were prepared and used to deplete PBMC from coeliac subjects on day 6 or 7 after commencing 3 day gluten challenge. FACS analysis demonstrated α^E beads depleted approximately 50% of positive CD4 T cells, while β_7 beads depleted all β_7 positive CD4 T cells. Depletion of PBMC using 10 CD4- or β_7 -beads, but not CD8- or α^E -beads, abolished responses in the interferon gamma ELISpot. tTG gliadin and PPD responses were abolished by CD4 depletion, but consistently affected by integrin-specific bead depletion.

Thus A-gliadin 57-73 QE65-specific T cells induced after gluten challenge in coeliac disease express the integrin, $\alpha_4\beta_7$, present on lamina propria CD4 T cells in 15 the small intestine.

Example 5

Optimal T cell Epitope Length

Previous data testing peptides from 7 to 17 amino acids in length spanning 20 the

core of the dominant T cell epitope in A-gliadin indicated that the 17mer, A-gliadin 57-73 QE65 (SEQ ID NO:2) induced maximal responses in the interferon gamma Elispot using peripheral blood mononuclear cells (PBMC) from coeliac volunteers 6 days after commencing a 3-day gluten challenge.

25 Peptides representing expansions form the core sequence of the dominant T cell epitope in A-gliadin were assessed in the IFN gamma ELISPOT using peripheral blood mononuclear cells (PBMC) from coeliac volunteers in 6 days after commencing a 3-day gluten challenge (n=4). Peptide 13: A-gliadin 59-71 QE65 (13mer), peptide 15: 58-72 QE65 (15mer), ..., peptide 27: 52-78 SE65 (27mer).

30 As shown in Figure 11 expansion of the A-gliadin 57-73 QE65 sequence does not substantially enhance response in the IFN γ Elispot. Subsequent Examples

characterise the agonist and antagonist activity of A-gliadin 57-73 QE65 using 17mer peptides.

Example 6

5 *Comparison of A-gliadin 57-73 QE65 with other DQ2-restricted T cell epitopes in coeliac disease*

Dose response studies were performed using peptides corresponding to unmodified and transglutaminase-treated peptides corresponding to T cell epitopes of gluten-specific T cell clones and lines from intestinal biopsies of coeliac subjects.

10 Responses to peptides were expressed as percent of response to A-gliadin 57-73 QE65. All subjects were HLA-DQ2+ (none were DQ8+).

The studies indicate that A-gliadin 57-73 QE65 is the most potent gliadin peptide for induction of interferon gamma in the ELISpot assay using coeliac PBMC after gluten challenge (see Figure 12a-h, and Tables 5 and 6). The second and third 15 epitopes are suboptimal fragments of larger peptides i.e. A-gliadin 57-73 QE65 and GDA4_WHEAT P04724-84-100 QE92. The epitope is only modestly bioactive (approximately 1/20th as active as A-gliadin 57-73 QE65 after blank is subtracted).

20 A-gliadin 57-73 QE65 is more potent than other known T cell epitopes in coeliac disease. There are 16 polymorphisms of A-gliadin 57-73 (including the sequence PQLPY (SEQ ID NO:12)) amongst sequenced gliadin genes, their bioactivity is assessed next.

Example 7

25 *Comparison of gliadin- and A-gliadin 57-73 QE65-specific responses in peripheral blood*

The relative contribution of the dominant epitope, A-gliadin 57-73 QE65, to the total T cell response to gliadin in coeliac disease is a critical issue. Pepsin-trypsin and chymotrypsin-digested gliadin have been traditionally used as antigen for development of T cell lines and clones in coeliac disease. However, it is possible 30 that these proteases may cleave through certain peptide epitopes. Indeed, chymotrypsin digestion of recombinant α 9-gliadin generates the peptide QLQPFPQPELPY (SEQ ID NO:13), that is a truncation of the optimal epitope

sequence QLQPFPQPELPYPQPQS (SEQ ID NO:2) (see above).

Transglutaminase-treatment substantially increases the potency of chymotrypsin-digested gliadin in proliferation assays of gliadin-specific T cell clones and lines.

Hence, transglutaminase-treated chymotrypsin-digested gliadin (tTG gliadin) may

5 not be an ideal antigen, but responses against this mixture may approximate the "total" number of peripheral blood lymphocyte specific for gliadin. Comparison of responses against A-gliadin 57-73 QE65 and tTG gliadin in the ELISpot assay gives an indication of the contribution of this dominant epitope to the overall immune response to gliadin in coeliac disease, and also be a measure of epitope spreading.

10 PBMC collected on day 6 or 7 after commencing gluten challenge in 4 coeliac subjects were assessed in dose response studies using chymotrypsin-digested gliadin +/- tTG treatment and compared with ELISpot responses to an optimal concentration of A-gliadin 57-73 QE65 (25mcg/ml). TTG treatment of gliadin enhanced PBMC responses in the ELISpot approximately 10-fold (tTG was 15 comparable to blank when assessed alone) (see Figure 13a-c). In the four coeliac subjects studied, A-gliadin 57-73 QE65 (25 mcg/ml) elicited responses between 14 and 115% those of tTG gliadin (500 mcg/ml), and the greater the response to A-gliadin 57-73 QE65 the greater proportion it represented of the tTG gliadin response.

Relatively limited data suggest that A-gliadin 57-73 QE65 responses are 20 comparable to tTG gliadin in some subjects. Epitope spreading associated with more evolved anti-gliadin T cell responses may account for the smaller contribution of A-gliadin 57-73 QE65 to "total" gliadin responses in peripheral blood in some individuals. Epitope spreading may be maintained in individuals with less strictly gluten free diets.

25

Example 8

Definition of gliadin peptides bioactive in coeliac disease: polymorphisms of A-gliadin 57-73

Overlapping 15mer peptides spanning the complete sequence of A-gliadin 30 were assessed in order to identify the immunodominant sequence in coeliac disease. A-gliadin was the first fully sequenced alpha gliadin protein and gene, but is one of approximately 30-50 related alpha gliadin proteins in wheat. Twenty five distinct

alpha-gliadin genes have been identified by searching protein data bases, Swiss-Prot and TREMBL describing a further 8 alpha-gliadins. Contained within these 25 alpha-gliadins, there are 16 distinct polymorphisms of the sequence corresponding to A-gliadin 57-73 (see Table 7).

5 Synthetic peptides corresponding to these 16 polymorphisms, in an unmodified form, after treatment with transglutaminase in vitro, as well as with glutamate substituted at position 10 (equivalent to QE65 in A-gliadin 57-73) were assessed using PBMC from coeliac subjects, normally following a gluten free diet, day 6 or 7 after gluten challenge in interferon gamma ELISpot assays. Glutamate-
10 substituted peptides were compared at three concentrations (2.5, 25 and 250 mcg/ml), unmodified peptide and transglutaminase-treated peptides were assessed at 25 mcg/ml only. Bioactivity was expressed as % of response associated with A-gliadin 57-73 QE65 25 mcg/ml in individual subjects (n=4). (See Fig 14).

Bioactivity of "wild-type" peptides was substantially increased (>5-fold) by
15 treatment with transglutaminase. Transglutaminase treatment of wild-type peptides resulted in bioactivity similar to that of the same peptides substituted with glutamate at position 10. Bioactivities of five glutamate-substituted peptides (B, C, K, L, M), were >70% that of A-gliadin 57-73 QE65 (A), but none was significantly more bioactive than A-gliadin 57-73 QE65. PBMC responses to glutamate-substituted
20 peptides at concentrations of 2.5 and 250 mcg/ml were comparable to those at 25 mcg/ml. Six glutamate-substituted gliadin peptides (H, I, J, N, O, P) were <15% as bioactive as A-gliadin 57-73 QE65. Other peptides were intermediate in bioactivity.

At least six gliadin-derived peptides are equivalent in potency to A-gliadin 57-73 QE65 after modification by transglutaminase. Relatively non-bioactive
25 polymorphisms of A-gliadin 57-73 also exist. These data indicate that transglutaminase modification of peptides from several gliadins of *Triticum aestivum*, *T. uartu* and *T. spelta* may be capable of generating the immunodominant T cell epitope in coeliac disease.

Genetic modification of wheat to generate non-coeliac-toxic wheat may likely
30 require removal or modification of multiple gliadin genes. Generation of wheat containing gliadins or other proteins or peptides incorporating sequences defining altered peptide ligand antagonists of A-gliadin 57-73 is an alternative strategy to

generate genetically modified wheat that is therapeutic rather than "non-toxic" in coeliac disease.

Example 9

5 *Definition of Core Epitope Sequence:*

Comparison of peptides corresponding to truncations of A-gliadin 56-75 from the N- and C-terminal indicated that the core sequence of the T cell epitope is PELPY (A-gliadin 64-68). Attempts to define non-agonists and antagonists will focus on variants of A-gliadin that are substituted at residues that substantially 10 contribute to its bioactivity.

Peptides corresponding to A-gliadin 57-73 QE65 with alanine (Figure 15) or lysine (Figure 16) substituted for residues 57 to 73 were compared in the IFN gamma ELISPOT using peripheral blood mononuclear cells (PBMC) from coeliac volunteers 6 days after commencing a 3-day gluten challenge (n=8). (BL is blank, E is A-gliadin 57-73 QE65: QLQPFPQPELPYPQPQS (SEQ ID NO:2)).

It was found that residues corresponding to A-gliadin 60-70 QE65 (PFPQPELPYPQ (SEQ ID NO:14)) contribute substantially to the bioactivity in A-gliadin 57-73 QE65. Variants of A-gliadin 57-73 QE65 substituted at positions 60-70 are assessed in a 2-step procedure. Initially, A-gliadin 57-73 QE65 substituted at 20 positions 60-70 using 10 different amino acids with contrasting properties are assessed. A second group of A-gliadin 57-73 QE65 variants (substituted with all other naturally occurring amino acids except cysteine at positions that prove are sensitive to modification) are assessed in a second round.

25 **Example 10**

Agonist activity of substituted variants of A-gliadin 57-73 QE65

A-gliadin 60-70 QE65 is the core sequence of the dominant T cell epitope in A-gliadin. Antagonist and non-agonist peptide variants of this epitope are most likely generated by modification of this core sequence. Initially, A-gliadin 57-73 30 QE65 substituted at positions 60-70 using 10 different amino acids with contrasting properties will be assessed in the IFNgamma ELISPOT using PBMC from coeliac subjects 6 days after starting 3 day gluten challenge. A second group of A-gliadin

57-73 QE65 variants (substituted with all other naturally occurring amino acids except cysteine) at positions 61-70 were also assessed. Both groups of peptides (all at 50 mcg/ml, in duplicate) were assessed using PBMC from 8 subjects and compared to the unmodified peptide (20 replicates per assay). Previous studies 5 indicate that the optimal concentration for A-gliadin 57-73 QE65 in this assay is between 10 and 100 mcg/ml.

Results are expressed as mean response in spot forming cells (95% confidence interval) as % A-G 57-73 QE65 mean response in each individual. Unpaired t-tests will be used to compare ELISPOT responses of modified peptides 10 with A-G 57-73 QE65. Super-agonists were defined as having a greater response than A-G 57-73 QE65 at a level of significance of $p<0.01$; partial agonists as having a response less than A-G 57-73 QE65 at a level of significance of $p<0.01$, and non-agonists as being not significantly different ($p>0.01$) from blank (buffer without peptide). Peptides with agonist activity 30% or less than that of A-gliadin 57-73 QE65 15 were considered "suitable" partial or non-agonists to assess for antagonistic activity (see Table 8 and Figures 17-27).

The IFNgamma ELISPOT response of PBMC to A-gliadin 57-73 QE65 is highly specific at a molecular level. Proline at position 64 (P64), glutamate at 65 (E65), and leucine at position 66 (L66), and to a lesser extent Q63, P67, Y68 and P69 20 are particularly sensitive to modification. The substitutions Y61 and Y70 both generate super-agonists with 30% greater bioactivity than the parent peptide, probably by enhancing binding to HLA-DQ2 since the motif for this HLA molecule indicates a preference for bulky hydrophobic residues at positions 1 and 9. Eighteen non-agonist peptides were identified. Bioactivities of the variants (50 mcg/ml): P65, 25 K64, K65 and Y65 (bioactivity 7-8%) were comparable to blank (7%). In total, 57 mutated variants of A-gliadin 57-73 QE65 were 30% or less bioactive than A-gliadin 57-73 QE65.

The molecular specificity of the peripheral blood lymphocyte (PBL) T cell response to the dominant epitope, A-gliadin 57-73 QE65, is consistently reproducible 30 amongst HLA-DQ2+ coeliac subjects, and is highly specific to a restricted number of amino acids in the core 7 amino acids. Certain single-amino acid variants of A-gliadin 57-73 QE65 are consistently non-agonists in all HLA-DQ2+ coeliac subjects.

Example 11*Antagonist activity of substituted variants*

The homogeneity of the PBL T cell response to A-gliadin 57-73 QE65 in HLA-DQ2+ coeliac disease suggests that altered peptide ligands (APL) capable of antagonism in PBMC ex vivo may exist, even though the PBL T cell response is likely to be poly- or oligo-clonal. APL antagonists are generally weak agonists.

5 Fifty-seven single amino acid-substituted variants of A-gliadin 57-73 QE65 with agonist activity 30% or less have been identified and are suitable candidates as APL antagonists. In addition, certain weakly bioactive naturally occurring polymorphisms of A-gliadin 57-73 QE65 have also been identified (see below) and may be "naturally occurring" APL antagonists. It has also been suggested that competition for binding MHC may also antagonise antigen-specific T cell immune. Hence, non-gliadin peptides that do not induce IFNgamma responses in coeliac PBMC after

10 gluten challenge but are known to bind to HLA-DQ2 may be capable of reducing T cell responses elicited by A-gliadin 57-73 QE65. Two peptides that bind avidly to HLA-DQ2 are HLA class 1 α 46-60 (HLA 1a) (PRAPWIEQEGPEYW (SEQ ID NO:15)) and thyroid peroxidase (tp) 632-645Y (IDVWLGGLLAENFLPY (SEQ ID NO:16)).

15 Simultaneous addition of peptide (50 μ g/ml) or buffer and A-gliadin 57-73 QE65 (10 μ g/ml) in IFNgamma ELISPOT using PBMC from coeliac volunteers 6 days after commencing 3 day gluten challenge (n=5). Results were expressed as response with peptide plus A-G 57-73 QE65 (mean of duplicates) as % response with buffer plus A-G 57-73 QE65 (mean of 20 replicates). (See Table 9).

20 Four single amino acid-substituted variants of A-gliadin 57-73 QE65 reduce the interferon gamma PBMC ELISPOT response to A-gliadin 57-73 QE65 ($p<0.01$) by between 25% and 28%, 13 other peptide variants reduce the ELISPOT response by between 18% and 24% ($p<0.06$). The HLA-DQ2 binder, thyroid peroxidase (tp) 632-645Y reduces PBMC interferon gamma responses to A-gliadin 57-73 QE65 by

25 31% ($p<0.0001$) but the other HLA-DQ2 binder, HLA class 1 α 46-60, does not alter responses (see Tables 9 and 10). The peptide corresponding to a transglutaminase-modified polymorphism of A-gliadin 57-73, SwissProt accession no.: P04725' 82-98

QE90 (PQPQPFPPPELPYPQPQS (SEQ ID NO:17)) reduces responses to A-gliadin 57-73 QE65 by 19% (p<0.009) (see Table 11).

Interferon gamma responses of PBMC to A-gliadin 57-73 QE65 in ELISPOT assays are reduced by co-administration of certain single-amino-acid A-gliadin 57-73

5 QE65 variants, a polymorphism of A-gliadin 57-73 QE65, and an unrelated peptide known to bind HLA-DQ2 in five-fold excess. These findings suggest that altered peptide ligand antagonists of A-gliadin 57-73 QE65 exist. Not only putative APL antagonists but also certain peptides that bind HLA-DQ2 effectively reduce PBL T cell responses to A-gliadin 57-73 QE65.

10 These findings support two strategies to interrupt the T cell response to the dominant A-gliadin epitope in HLA-DQ2+ coeliac disease.

1. Optimisation of APL antagonists by substituting amino acids at more than one position (64-67) for use as "traditional" peptide pharmaceuticals or for specific genetic modification of gliadin genes in wheat.

15 2. Use of high affinity HLA-DQ2 binding peptides to competitively inhibit presentation of A-gliadin 57-73 QE65 in association with HLA-DQ2.

These two approaches may be mutually compatible. Super-agonists were generated by replacing F61 and Q70 with tyrosine residues. It is likely these super-
20 agonists resulted from improved binding to HLA-DQ2 rather than enhanced contact with the T cell receptor. By combining these modifications with other substitutions that generate modestly effective APL antagonists might substantially enhance the inhibitory effect of substituted A-gliadin 57-73 QE65 variants.

25 **Example 12**

Development of interferon gamma ELISpot using PBMC and A-gliadin 57-73 QE65 and P04724 84-100 QE92 as a diagnostic for coeliac disease: Definition of immune-responsiveness in newly diagnosed coeliac disease

Induction of responsiveness to the dominant A-gliadin T cell epitope in
30 PBMC measured in the interferon gamma ELISpot follows gluten challenge in almost all DQ2+ coeliac subjects following a long term strict gluten free diet (GFD) but not in healthy DQ2+ subjects after 4 weeks following a strict GFD. A-gliadin

57-73 QE65 responses are not measurable in PBMC of coeliac subjects before gluten challenge and pilot data have suggested these responses could not be measured in PBMC of untreated coeliacs. These data suggest that in coeliac disease immune-responsiveness to A-gliadin 57-73 QE65 is restored following antigen exclusion (GFD). If a diagnostic test is to be developed using the ELISpot assay and PBMC, it is desirable to define the duration of GFD required before gluten challenge is capable of inducing responses to A-gliadin 57-73 QE65 and other immunoreactive gliadin peptides in blood.

10 Newly diagnosed DQ2+ coeliac subjects were recruited from the gastroenterology outpatient service. PBMC were prepared and tested in interferon gamma ELISpot assays before subjects commenced GFD, and at one or two weeks after commencing GFD. In addition, gluten challenge (3 days consuming 4 slices standard white bread, 200g/day) was performed at one or two weeks after starting GFD. PBMC were prepared and assayed on day six are after commencing gluten challenge. A-gliadin 57-73 QE65 (A), P04724 84-100 QE92 (B) (alone and combined) and A-gliadin 57-73 QP65 (P65) (non-bioactive variant, see above) (all 25 mcg/ml) were assessed.

15 All but one newly diagnosed coeliac patient was DQ2+ (one was DQ8+) (n=11). PBMC from newly diagnosed coeliacs that were untreated, or after 1 or 2 weeks following GFD did not show responses to A-gliadin 57-73 QE65 and P04724 84-100 QE92 (alone or combined) that were not significantly different from blank or A-gliadin 57-73 QP65 (n=9) (see Figure 28). Gluten challenge in coeliacs who had followed GFD for only one week did not substantially enhance responses to A-gliadin 57-73 QE65 or P04724 84-100 QE92 (alone or combined). But gluten challenge 2 weeks after commencing GFD did induce responses to A-gliadin 57-73 QE65 and P04724 84-100 QE92 (alone or combined) that were significantly greater than the non-bioactive variant A-gliadin 57-73 QP65 and blank. Although these responses after gluten challenge at 2 weeks were substantial they appear to be less than in subjects >2 months after commencing GFD. Responses to A-gliadin 57-73 QE65 alone were equivalent or greater than responses to P04724 84-100 QE92 alone or when mixed with A-gliadin 57-73 QE65. None of the subjects experienced troubling symptoms with gluten challenge.

Immune responsiveness (as measured in PBMC after gluten challenge) to A-gliadin is partially restored 2 weeks after commencing GFD, implying that "immune unresponsiveness" to this dominant T cell epitope prevails in untreated coeliac disease and for at least one week after starting GFD. The optimal timing of a 5 diagnostic test for coeliac disease using gluten challenge and measurement of responses to A-gliadin 57-73 QE65 in the ELISpot assay is at least 2 weeks after commencing a GFD.

Interferon gamma-secreting T cells specific to A-gliadin 57-73 QE65 cannot be measured in the peripheral blood in untreated coeliacs, and can only be induced 10 by gluten challenge after at least 2 weeks GFD (antigen exclusion). Therefore, timing of a diagnostic test using this methodology is crucial and further studies are needed for its optimization. These finding are consistent with functional anergy of T cells specific for the dominant epitope, A-gliadin 57-73 QE65, reversed by antigen exclusion (GFD). This phenomenon has not been previously demonstrated in a 15 human disease, and supports the possibility that T cell anergy may be inducible with peptide therapy in coeliac disease.

Example 13

Comprehensive Mapping of Wheat Gliadin T Cell Epitopes

20 Antigen challenge induces antigen-specific T cells in peripheral blood. In coeliac disease, gluten is the antigen that maintains this immune-mediated disease. Gluten challenge in coeliac disease being treated with a gluten free diet leads to the appearance of gluten-specific T cells in peripheral blood, so enabling determination of the molecular specificity of gluten T cell epitopes. As described above, we have 25 identified a single dominant T cell epitope in a model gluten protein, A-gliadin (57-73 deamidated at Q65). In this Example, gluten challenge in coeliac patients was used to test all potential 12 amino acid sequences in every known wheat gliadin protein derived from 111 entries in Genbank. In total, 652 20mer peptides were tested in HLA-DQ2 and HLA-DQ8 associated coeliac disease. Seven of the 9 30 coeliac subjects with the classical HLA-DQ2 complex (HLA-DQA1*05, HLA-DQB1*02) present in over 90% of coeliacs had an inducible A-gliadin 57-73 QE65- and gliadin-specific T cell response in peripheral blood. A-gliadin 57-73 was the

only significant α -gliadin T cell epitope, as well as the most potent gliadin T cell epitope, in HLA-DQ2-associated coeliac disease. In addition, there were as many as 5 families of structurally related peptides that were between 10 and 70% as potent as A-gliadin 57-73 in the interferon- γ ELISpot assay. These new T cell epitopes were 5 derived from γ - and ω -gliadins and included common sequences that were structurally very similar, but not identical to the core sequence of A-gliadin 57-73 (core sequence: FPQPQLPYP (SEQ ID NO:18)), for example: FPQPQQPFP (SEQ ID NO:19) and PQQPQQPFP (SEQ ID NO:20). Although no homologues of A-gliadin 57-73 have been found in rye or barley, the other two cereals toxic in coeliac 10 disease, the newly defined T cell epitopes in γ - and ω -gliadins have exact matches in rye and barley storage proteins (secalins and hordeins, respectively).

Coeliac disease not associated with HLA-DQ2 is almost always associated with HLA-DQ8. None of the seven HLA-DQ8+ coeliac subjects had inducible A-gliadin 57-73-specific T cell responses following gluten challenge, unless they also 15 possessed the complete HLA-DQ2 complex. Two of 4 HLA-DQ8+ coeliac subjects who did not possess the complete HLA-DQ2 complex, had inducible gliadin peptide-specific T cell responses following gluten challenge. In one HLA-DQ8 subject, a novel dominant T cell epitope was identified with the core sequence LQPQNPSPQQPQ (SEQ ID NO:21). The transglutaminase-deamidated version of 20 this peptide was more potent than the non-deamidated peptide. Previous studies suggest that the transglutaminase-deamidated peptide would have the sequence LQPENPSSEQPE (SEQ ID NO:22); but further studies are required to confirm this sequence. Amongst the healthy HLA-DQ2 (10) and HLA-DQ8 (1) subjects who followed a gluten free diet for a month, gliadin peptide-specific T cell responses 25 were uncommon, seldom changed with gluten challenge, and were never potent T cell epitopes revealed with gluten challenge in coeliac subjects. In conclusion, there are unlikely to be more than six important T cell epitopes in HLA-DQ2-associated coeliac disease, of which A-gliadin 57-73 is the most potent. HLA-DQ2- and HLA-DQ8-associated coeliac disease do not share the same T cell specificity.

30 We have shown that short-term gluten challenge of individuals with coeliac disease following a gluten free diet induces gliadin-specific T cells in peripheral blood. The frequency of these T cells is maximal in peripheral blood on day 6 and

then rapidly wanes over the following week. Peripheral blood gliadin-specific T cells express the integrin $\alpha 4\beta 7$ that is associated with homing to the gut lamina propria. We exploited this human antigen-challenge design to map T cell epitopes relevant to coeliac disease in the archetypal gluten α -gliadin protein, A-gliadin.

5 Using 15mer peptides overlapping by 10 amino acids with and without deamidation by transglutaminase (tTG), we demonstrated that T cells induced in peripheral blood initially target only one A-gliadin peptide, residues 57-73 in which glutamine at position 65 is deamidated. The epitope is HLA-DQ2-restricted, consistent with the intimate association of coeliac disease with HLA-DQ2.

10 Coeliac disease is reactivated by wheat, rye and barley exposure. The α/β -gliadin fraction of wheat gluten is consistently toxic in coeliac disease, and most studies have focused on these proteins. The gene cluster coding for α/β -gliadins is located on wheat chromosome 6C. There are no homologues of α/β -gliadins in rye or barley. However, all three of the wheat gliadin subtypes (α/β , γ , and ω) are toxic 15 in coeliac disease. The γ - and ω -gliadin genes are located on chromosome 1A in wheat, and are homologous to the secalins and hordeins in rye and barley.

There are now genes identified for 61 α -gliadins in wheat (*Triticum aestivum*). The α -gliadin sequences are closely homologous, but the dominant epitope in A-gliadin derives from the most polymorphic region in the α -gliadin 20 sequence. Anderson et al (1997) have estimated that there are a total of about 150 distinct α -gliadin genes in *T. aestivum*, but many are pseudogenes. Hence, it is unlikely that T-cell epitopes relevant to coeliac disease are not included within known α -gliadin sequences.

Our work has identified a group of deamidated α -gliadin peptides almost 25 identical to A-gliadin 57-73 as potent T cell epitopes specific to coeliac disease. Over 90% of coeliac patients are HLA-DQ2+, and so far, we have only assessed HLA-DQ2+ coeliac subjects after gluten challenge. However, coeliac patients who do not express HLA-DQ2 nearly all carry HLA-DQ8. Hence, it is critical to know whether A-gliadin 57-73 and its homologues in other wheat, rye and barley gluten 30 proteins are the only T-cell epitopes recognized by T cells induced by gluten challenge in both HLA-DQ2+ and HLA-DQ8+ coeliac disease. If this were the case, design of peptide therapeutics for coeliac disease might only require one peptide.

Homologues of A-gliadin 57-73 as T-cell epitopes

Initial searches of SwissProt and Trembl gene databases for cereal genes coding for the core sequence of A-gliadin 57-73 (PQLPY <SEQ ID NO:12>) only 5 revealed α/β -gliadins. However, our fine-mapping studies of the A-gliadin 57-73 QE65 epitope revealed a limited number of permissive point substitutions in the core region (PQLP) (note Q65 is actually deamidated in the epitope). Hence, we extended our search to genes in SwissProt or Trembl databases encoding for peptides with the sequence XXXXXXXXPQ[ILMP][PST]XXXXXX (SEQ ID NO:23). Homologues 10 were identified amongst γ -gliadins, glutenins, hordeins and secalins (see Table 12). A further homologue was identified in ω -gliadin by visual search of the three ω -gliadin entries in Genbank.

These homologues of A-gliadin 57-73 were assessed after deamidation by tTG (or synthesis of the glutamate(QE)-substituted variant in four close homologues) 15 using the IFN γ ELISpot assay with peripheral blood mononuclear cells after gluten challenge in coeliac subjects. The ω -gliadin sequence (AAG17702 141-157) was the only bioactive peptide, approximately half as potent as A-gliadin 57-73 (see Table 12, and Figure 29). Hence, searches for homologues of the dominant A-gliadin epitope failed to account for the toxicity of γ -gliadin, secalins, and hordeins.

20 **Methods***Design of a set of peptides spanning all possible wheat gliadin T-cell epitopes*

In order to identify all possible T cell epitopes coded by the known wheat (Triticum aestivum) gliadin genes or gene fragments (61 α/β -, 47 γ -, and 3 ω -gliadin entries in Genbank), gene-derived protein sequences were aligned using the CystalW 25 software (MegAlign) and arranged into phylogenetic groupings (see Table 22):

Many entries represented truncations of longer sequences, and many gene segments were identical except for the length of polyglutamine repeats or rare substitutions. Hence, it was possible to rationalize all potential unique 12 amino acid sequences encoded by known wheat genes to be included in a set of 652 20mer peptides.

30 (Signal peptide sequences were not included). Peptide sequences are listed in Table 23.

Comprehensive epitope mapping

Healthy controls (HLA-DQ2+ n=10, and HLA-DQ8+ n=1) who had followed a gluten free diet for 4 weeks, and coeliac subjects (six HLA-DQ2, four complex heterozygotes HLA-DQ2/8, and three HLA-DQ8/X) (see Table 13) following long-term gluten free diet were studied before and on day 6 and 7 after 3-day gluten challenge (four 50g slices of standard white bread – Sainsbury's sandwich bread, each day). Peripheral blood (a total of 300ml over seven days) was collected and peripheral blood mononuclear cells (PBMC) were separated by Lymphoprep density gradient. PBMC were incubated with pools of 6 or 8 20mer peptides, or single peptides with or without deamidation by tTG in overnight interferon gamma (IFN γ)

10 ELISpot assays.

Peptides were synthesized in batches of 96 as Pepsets (Mimotopes Inc., Melbourne Australia). Approximately 0.6 micromole of each of 652 20mers was provided. Two marker 20mer peptides were included in each set of 96 (VLQQQHNIAHGSSQVLQESTY – peptide 161 (SEQ ID NO:24), and

15 IKDFHVYFRESRDALWKPG (SEQ ID NO:25)) and were characterized by reverse phase-HPLC and amino acid sequence analysis. Average purities of these marker peptides were 50% and 19%, respectively. Peptides were initially dissolved in acetonitrile (10%) and Hepes 100mM to 10mg/ml.

The final concentration of individual peptides in pools (or alone) incubated with PBMC for the IFN γ ELISpot assays was 20 μ g/ml. Five-times concentrated solutions of peptides and pools in PBS with calcium chloride 1mM were aliquotted and stored in 96-well plates according to the template later used in ELISpot assays. Deamidated peptides and pools of peptides were prepared by incubation with guinea pig tissue tTG (Sigma T5398) in the ratio 100:32 μ g/ml for two hours at 37°C.

25 Peptides solutions were stored at -20°C and freshly thawed prior to use.

Gliadin (Sigma G3375) (100 mg/ml) in endotoxin-free water and 2M urea was boiled for 10 minutes, cooled to room temperature and incubated with filter (0.2 μ m)-sterilised pepsin (Sigma P6887) (2 mg/ml) in HCl 0.02M or chymotrypsin (C3142) (4mg/ml) in ammonium bicarbonate (0.2M). After incubation for 4 hours,

30 pepsin-digested gliadin was neutralized with sodium hydroxide, and then both pepsin- and chymotrypsin-digested gliadin were boiled for 15 minutes. Identical incubations with protease in which gliadin was omitted were also performed.

Samples were centrifuged at 15 000g, then protein concentrations were estimated in supernatants by the BCA method (Pierce, USA). Before final use in IFN γ ELISpot assays, aliquots of gliadin-protease were incubated with tTG in the ratio 2500:64 μ g/ml.

5 IFN γ ELISpot assays (Mabtech, Sweden) were performed in 96-well plates (MAIP S-45, Millipore) in which each well contained 25 μ l of peptide solution and 100 μ l of PBMC (2-8x10⁵/well) in RPMI containing 10% heat inactivated human AB serum. Deamidated peptide pools were assessed in one 96-well ELISpot plate, and peptides pools without deamidation in a second plate (with an identical layout) on 10 both day 0 and day 6. All wells in the plate containing deamidated peptides included tTG (64 μ g/ml). In each ELISpot plate there were 83 wells with peptide pools (one unique pool in each well), and a series of wells for "control" peptides (peptides all >90% purity, characterized by MS and HPLC, Research Genetics): P04722 77-93 (QLQPFPQPQLPYPQPQP (SEQ ID NO:26)), P04722 77-93 QE85 (in duplicate) (QLQPFPQPQLPYPQPQP (SEQ ID NO:27)), P02863 77-93 (QLQPFPQPQLPYSQPQP (SEQ ID NO:28)), P02863 77-93 QE85 (QLQPFPQPQLPYSQPQP (SEQ ID NO:29)), and chymotrypsin-digested gliadin (500 μ g/ml), pepsin-digested gliadin (500 μ g/ml), chymotrypsin (20 μ g/ml) alone, pepsin (10 μ g/ml) alone, and blank (PBS+/-tTG) (in triplicate).

15 20 After development and drying, IFN γ ELISpot plates were assessed using the MAIP automated ELISpot plate counter. In HLA-DQ2 healthy and coeliac subjects, induction of spot forming cells (sfc) by peptide pools in the IFN γ ELISpot assay was tested using a one-tailed Wilcoxon Matched-Pairs Signed-Ranks test (using SPSS software) applied to spot forming cells (sfc) per million PBMC minus blank on day 6 25 versus day 0 ("net response"). Significant induction of an IFN γ response to peptide pools in PBMC by *in vivo* gluten challenge was defined as a median "net response" of at least 10 sfc/million PBMC and p<0.05 level of significance. Significant response to a particular pool of peptides on day 6 was followed by assessment of individual peptides within each pool using PBMC drawn the same day or on day 7.

30 30 For IFN γ ELISpot assays of individual peptides, bioactivity was expressed as a percent of response to P04722 77-93 QE85 assessed in the same ELISpot plate. Median response to blank (PBS alone) was 0.2 (range 0-5) sfc per well, and the

positive control (P04722 77-93 QE85) 76.5 (range: 25-282) sfc per well using a median of 0.36 million (range: 0.3-0.72) PBMC. Hence, median response to blank expressed as a percentage of P04722 77-93 QE65 was 0.2% (range: 0-6.7). Individual peptides with mean bioactivity greater than 10% that of P04722 QE85 5 were analyzed for common structural motifs.

Results

Healthy HLA-DQ2 subjects

None of the healthy HLA-DQ2+ subjects following a gluten free diet for a month had IFN γ ELISpot responses to homologues of A-gliadin 57-73 before or after 10 gluten challenge. However, in 9/10 healthy subjects, gluten challenge was associated with a significant increase in IFN γ responses to both peptic- and chymotryptic-digests of gliadin, from a median of 0-4 sfc/million on day 0 to a median of 16-29 sfc/million (see Table 14). Gliadin responses in healthy subjects were unaffected by deamidation (see Table 15). Amongst healthy subjects, there was no consistent 15 induction of IFN γ responses to specific gliadin peptide pools with gluten challenge (see Figure 30, and Table 16). IFN γ ELISpot responses were occasionally found, but these were weak, and not altered by deamidation. Many of the strongest responses to pools were also present on day 0 (see Table 17, subjects H2, H8 and H9). Four healthy subjects did show definite responses to pool 50, and the two with strongest 20 responses on day 6 also had responses on day 0. In both subjects, the post-challenge responses to pool 50 responses were due to peptide 390 (QQTYPQRPQQPFPQTQQPQQ (SEQ ID NO:30)).

HLA-DQ2 coeliac subjects

Following gluten challenge in HLA-DQ2+ coeliac subjects, median IFN γ 25 ELISpot responses to P04722 77-93 E85 rose from a median of 0 to 133 sfc/million (see Table 4). One of the six coeliac subjects (C06) did not respond to P04722 77-93 QE85 (2 sfc/million) and had only weak responses to gliadin peptide pools (maximum: Pool 50+ α TG 27 sfc/million). Consistent with earlier work, bioactivity of wild-type P04722 increased 6.5 times with deamidation by α TG (see Table 15). 30 Interferon-gamma responses to gliadin-digests were present at baseline, but were substantially increased by gluten challenge from a median of 20 up to 92 sfc/million for chymotryptic-gliadin, and from 44 up to 176 sfc/million for peptide-gliadin.

Deamidation of gliadin increased bioactivity by a median of 3.2 times for chymotryptic-gliadin and 1.9 times for peptic-gliadin (see Table 15). (Note that the acidity required for digestion by pepsin is likely to result in partial deamidation of gliadin.)

5 In contrast to healthy subjects, gluten challenge induced IFN γ ELISpot responses to 22 of the 83 tTG-treated pools including peptides from α -, γ - and ω -gliadins (see Figure 31, and Table 17). Bioactivity of pools was highly consistent between subjects (see Table 18). IFN γ ELISpot responses elicited by peptide pools were almost always increased by deamidation (see Table 17). But enhancement of 10 bioactivity of pools by deamidation was not as marked as for P04722 77-73 Q85, even for pools including homologues of A-gliadin 57-73. This suggests that Pepset peptides were partially deamidated during synthesis or in preparation, for example the Pepset peptides are delivered as salts of trifluoroacetic acid (TFA) after lyophilisation from a TFA solution.

15 One hundred and seventy individual tTG-deamidated peptides from 21 of the most bioactive pools were separately assessed. Seventy-two deamidated peptides were greater than 10% as bioactive as P04722 77-93 QE85 at an equivalent concentration (20 μ g/ml) (see Table 19). The five most potent peptides (85-94% bioactivity of P04722 QE85) were previously identified α -gliadin homologues A- 20 gliadin 57-73. Fifty of the bioactive peptides were not homologues of A-gliadin 57-73, but could be divided into six families of structurally related sequences (see Table 20). The most bioactive sequence of each of the peptide families were: PQQPQQPQQPFPOPOQQFPW (SEQ ID NO:31) (peptide 626, median 72% bioactivity of P04722 QE85), QQPQQPFPOPOQQPOLPFPQQ (SEQ ID NO:32) (343, 34%), QAFPOPOQQTFPHQPOQQFPQ (SEQ ID NO:33) (355, 27%), TQQPQQPFPOQQPFPQTQ (SEQ ID NO:34) (396, 23%), PIQPOOPFPQQPQQPQQFP (SEQ ID NO:35) (625, 22%), PQQSFSYQQQPFPOQQYPQQ (SEQ ID NO:36) (618, 18%) (core sequences are underlined). All of these sequences include glutamine residues predicted to be 30 susceptible to deamidation by transglutaminase (e.g. QXP, QXPF (SEQ ID NO:37), QXX[FY] (SEQ ID NO:38)) (see Vader et al 2002). Some bioactive peptides contain two core sequences from different families.

Consistent with the possibility that different T-cell populations respond to peptides with distinct core sequences, bioactivity of peptides from different families appear to be additive. For example, median bioactivity of tTG-treated Pool 81 was 141% of P04722 QE85, while bioactivity of individual peptides was in rank order:

- 5 Peptide 631 (homologue of A-gliadin 57-73) 61%, 636 (homologue of 626) 51%, and 635 19%, 629 16%, and 634 13% (all homologues of 396).

Although likely to be an oversimplification, the contribution of each "peptide family" to the summed IFN γ ELISpot response to gliadin peptides was compared in the HLA-DQ2+ coeliac subjects (see Figure 32). Accordingly, the contribution of

- 10 P04722 77-73 E85 to the summed response to gliadin peptides is between 1/5 and 2/3.

Using the peptide homology search programme, WWW PepPepSearch, which can be accessed through the world wide web of the internet at, for example, "cbrg.inf.ethz.ch/subsection3_1_5.html.", and by direct comparison with Genbank sequences for rye secalins, exact matches were found for the core sequences QQFPFPQPQQPFP (SEQ ID NO:39) in barley hordeins (HOR8) and rye secalins (A23277, CAA26449, AAG35598), QQFPFPQPQQPFP (SEQ ID NO:40) in barley hordeins (HOG1 and HOR8), and for PIQPQQPFPQQP (SEQ ID NO:41) also in barley hordeins (HOR8).

20

HLA-DQ8-associated coeliac disease

Seven HLA-DQ8+ coeliac subjects were studied before and after gluten challenge. Five of these HLA-DQ8+ (HLA-DQA0*0301-3, HLA-DQB0*0302) subjects also carried one or both of the coeliac disease-associated HLA-DQ2 complex (DQA0*05, DQB0*02). Two of the three subjects with both coeliac-associated HLA-DQ complexes had potent responses to gliadin peptide pools (and individual peptides including P04722 77-93 E85) that were qualitatively and quantitatively identical to HLA-DQ2 coeliac subjects (see Figures 33 and 34, and Table 18). Deamidated peptide pool 74 was bioactive in both HLA-DQ2/8 subjects, but only in one of the 6 HLA-DQ2/X subjects. Pretreatment of pool 74 with tTG enhances bioactivity between 3.8 and 22-times, and bioactivity of tTG-treated pool 74 in the three responders is equivalent to between 78% and 350% the bioactivity of

P04722 77-93 E85. Currently, it is not known which peptides are bioactive in Pool 74 in subject C02, C07, and C08.

Two of the four HLA-DQ8 coeliac subjects that lacked both or one of the HLA-DQ2 alleles associated with coeliac disease showed very weak IFN γ ELISpot responses to gliadin peptide pools, but the other two did respond to both protease-digested gliadin and specific peptide pools. Subject C12 (HLA-DQ7/8) responded vigorously to deamidated Pools 1-3 (see Figure 35). Assessment of individual peptides in these pools identified a series of closely related bioactive peptides including the core sequence LQPQNPSQQQPQ (SEQ ID NO:42) (see Table 20).

10 Previous work (by us) has demonstrated that three glutamine residues in this sequence are susceptible to tTG-mediated deamidation (underlined). Homology searches using WWW PepPepSearch have identified close matches to LQPQNPSQQQPQ (SEQ ID NO:43) only in wheat α -gliadins.

The fourth HLA-DQ8 subject (C11) had inducible IFN γ ELISpot responses to tTG-treated Pool 33 (see Figure 36). Pools 32 and 33 include polymorphisms of a previously defined HLA-DQ8 restricted gliadin epitope (QQYPSGQGSFQPSQQNPQ (SEQ ID NO:44)) active after deamidation by tTG (underlined Gln are deamidated and convey bioactivity) (van der Wal et al 1998). Currently, it is not known which peptides are bioactive in Pool 33 in subject C11.

20 Comprehensive T cell epitope mapping in HLA-DQ2-associated coeliac disease using in vivo gluten challenge and a set of 652 peptides spanning all known 12 amino acid sequences in wheat gliadin has thus identified at least 72 peptides at 10% as bioactive as the known α -gliadin epitope, A-gliadin 57-73 E65. However, these bioactive peptides can be reduced to a set of perhaps as few as 5 distinct but 25 closely related families of peptides. Almost all these peptides are rich in proline, glutamine, phenylalanine, and/or tyrosine and include the sequence PQ(QL)P(FY)P (SEQ ID NO:45). This sequence facilitates deamidation of Q in position 2 by tTG. By analogy with deamidation of A-gliadin 57-68 (Arentz-Hansen 2000), the enhanced bioactivity of these peptides generally found with deamidation by tTG may 30 be due to increased affinity of binding for HLA-DQ2.

Cross-reactivity amongst T cells in vivo recognizing more than one of these bioactive gliadin peptides is possible. However, if each set of related peptides does

activate a distinct T cell population in vivo, the epitope corresponding to A-gliadin 57-73 E65 is the most potent and is generally recognized by at least 40% of the peripheral blood T cells that secrete IFN γ in response to gliadin after gluten challenge.

5. No gliadin-peptide specific responses were found in HLA-DQ2/8 coeliac disease that differed qualitatively from those in HLA-DQ2/X-associated coeliac disease. However, peripheral blood T cells in HLA-DQ8+ coeliac subjects without both HLA-DQ2 alleles did not recognize A-gliadin 57-73 E65 homologues. Two different epitopes were dominant in two HLA-DQ8+ coeliacs. The dominant epitope 10 in one of these HLA-DQ8+ individuals has not been identified previously (LQPQNPSQQQPQ (SEQ ID NO:46)).

Given the teaching herein, design of an immunotherapy for coeliac disease utilizing all the commonly recognised T cell epitopes is practical and may include fewer than six distinct peptides. Epitopes in wheat γ - and ω -gliadins are also present 15 in barley hordeins and rye secalins.

Example 14

Several ELISpot assays were performed as previously described and yielded the following results and/or conclusions:

20 *Examination of multiple α -gliadin polymorphisms with PQLPY*

Potent agonists of A-gliadin 57-73QE (G01) include QLQPFPQPELPYPQPQS (G01), PQL-Y—P (G10), and PQPQPFL— (G12). Less potent include —L—P (G04), —R—P (G05), and —S—P (G06). Less potent yet 25 include —L—S—P (G07), —S—S—P (G08), —S—S—P (G09), and PQPQPFP— (G13). Dashes indicate identity with the G01 sequence in the particular position.

30 *Gluten challenge induces A-gliadin 57-73 QE65 T cells only after two weeks of gluten-free diet in newly diagnosed coeliac disease*

Additional analyses indicated that tTG-deamidated gliadin responses change after two weeks of gluten-free diet in newly diagnosed coeliac disease. Other

analyses indicated that deamidated gliadin-specific T cells are $CD4^+ \alpha_4 \beta_7^+$ HLA-DQ2 restricted.

Optimal epitope (clones versus gluten challenge)

5 A "dominant" epitope is defined by γ IFN ELISpot after gluten challenge. QLQPFPQPELPPYQPQPS (100% ELISpot response). Epitopes defined by intestinal T cell clones: QLQPFPQPELPPY (27%), PQPELPPYQPQPELPPY (52%), and QQLPQPEQPQQSFPEQERPF (9%).

10

Dominance among individual peptide responses

15 Dominance depends on wheat or rye. For wheat, dominant peptides include peptide numbers 89, 90 and 91 (referring to sequence numbers in Table 23). For rye, dominant peptides include peptide numbers 368, 369, 370, 371, and 372 (referring to sequence numbers in Table 23). Some peptides, including 635 and 636 (referring to sequence numbers in Table 23) showed activity in both rye and wheat.

20 *In vivo gluten challenge allows T cell epitope hierarchy to be defined for coeliac disease*

The epitope hierarchy is consistent among HLA-DQ2 $^+$ coeliacs but different for HLA-DQ8 $^+$ coeliacs. The hierarchy depends on what cereal is consumed. Deamidation generates almost all gliadin epitopes. HLA-DQ2, DQ8, and DR4 present deamidated peptides. HLA-DQ2/8-associated coeliac disease preferentially present DQ2-associated gliadin epitopes. Gliadin epitopes are sufficiently restricted to justify development of epitope-based therapeutics.

Other analyses indicated the following: HLA-DR3-DQ2 (85-95%) and HLA-DR4-DQ8 (5-15%).

Other analyses indicated the following:

30	HLA-DQ	HLA-DQA1 allele	HLA-DQB1 allele	Duodenal histology	Gluten free	EMA on gluten (on GFD)
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C01	2,6	102/6, 501	201, 602	SVA	1 yr	+(-)	
C02	2,2	501	201	SVA	1 yr	+(-)	
C03	2,5	101/4/5, 501	201, 501	PVA	1 yr	+(-)	
C04	2,5	101/4/5, 501	201, 501	SVA	7 yr	+(-)	
5	C05	2,2	201, 501	201, 202	SVA	4 mo	+ (ND)
	C06	2,2	201, 501	201, 202	SVA	2 yr	+(-)
	C07	2,8	301-3, 501	201, 302	SVA	1 yr	+(-)
	C08	2,8	301-3, 501	201, 302/8	SVA	11 yr	ND (-)
	C09	2,8	301-3, 501	201, 302	SVA	29 yr	+(-)
10	C10	2,8	201, 301-3	202, 302	IEL	1 yr	+(-)
	C11	6,8	102/6, 301-3	602/15, 302/8	IEL	9 mo	- (ND)
	C12	8,7	301-3, 505	302, 301/9-10	SVA	2 yr	- (-)
	C13	8,8	301	302	SVA	1 yr	+ (+)

15 Another analysis was carried out to determine the bioactivity of individual tTG-deamidated peptides in pools 1-3 in subject C12. The results are as follows (sequence numbers refer to the peptides listed in Table 23): Sequence 8 (100%), Sequence 5 (85%), Sequence 6 (82%), Sequence 3 (77%), Sequence 1 (67%), Sequence 2 (59%), Sequence 9 (49%), Sequence 7 (49%), Sequence 10 (33%), Sequence 4 (15%), Sequence 12 (8%), Sequence 11 (0%), Sequence 23 (26%), Sequence 14 (18%), Sequence 15 (18%), Sequence 17 (18%), Sequence 16 (13%), Sequence 14 (8%), Sequence 22 (5%), Sequence 18 (3%), Sequence 19 (3%), Sequence 20 (0%), Sequence 21 (0%). The predicted deamidated sequence is LQPENPSSEQPE.

25

Individual ELISpot responses by PBMC (Spot forming cells determined by ELISpot Reader)

Peptide (see Table 23)	C01	C02	C03	C04	C05
65	16	2	1	2	3
30 66	32	6	13	0	6
67	16	3	4	0	4
68	25	8	4	2	2

69	4	0	0	0	0
70	2	1	0	0	0
71	1	1	0	0	1
72	0	0	0	0	0
73	95	21	42	31	31
74	122	15	29	21	28
75	5	1	2	2	5
76	108	13	28	16	22
77	3	0	1	0	1
78	21	2	3	5	3
79	20	0	2	0	2
80	5	2	0	0	3
81	4	1	2	3	1
82	3	3	5	2	2
83	14	2	0	0	1
84	3	0	0	0	0
85	14	1	2	1	2
86	11	0	2	0	2

20 *Cross-reactivity*

To deal with data from 652 peptides in 29 subjects, or to determine when a particular response is a true positive peptide-specific T-cell response, or to determine when a response to a peptide is due to cross-reactivity with another structurally related peptide, expression of a particular peptide response can be as a percentage of a "dominant" peptide response. Alternately, the expression can be a "relatedness" as correlation coefficients between peptide responses, or via bioinformatics.

Additional epitopes

30 A representative result is as follows:

Combination of peptides with P04722E (all 20mcg/ml) (n=4)

Alone	P04722E+
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Pep 626	60	135
P04722E	100	110
HLAa	0	85

(expressed as percent P04722E)

5 626+^tT: PQQPQQPQQPFPQPQQPFPW
P04724E: QLQPFPQPELPYPQPQL

TTG-deamidation of peptide 626 (n=12)

10 No ^tTG = 100%
TTG = 170%

Substitution at particular positions

Substitution of Peptide 626 PQQP[Q1]QP[Q2]QPFPQP[Q3]QPFPV (n=12)

15	Glu	Arg
Q1	95	90
Q2	145	80
Q3	155	10

(expressed as percent wild-type peptide)

20 Bioactivity of ^tTG-treated 15mers spanning Peptide 626/627
(PQQPQQPQQPFPQPQQPFPWQP) (n=8)

P1-15	5
P2-16	4
25 P3-17	3
P4-18	38
P5-19	65
P6-20	95
P7-21	65
30 P8-22	90

(expressed as percent of maximal 15mer response)

Multiple epitopes:

P04724E: QLQPFPQPQLPYPPQQL

626+TG: PQQPQQPQQPFPQPQQPFPW

Minimal epitope: QPQQPFPQPQQPFPW

5 Immunomagnetic depletion of PBMC by beads coated with anti-CD4 and by anti-integrin β_7 depleted IFN γ ELISpot responses, while immunomagnetic depletion of PBMC by beads coated with anti-CD8 or anti-alphaE integrin. Thus, the PBMC secreting IFN γ are CD4+ and $\alpha_4\beta_7+$, associated with homing to the lamina propria in the gut.

10 Blocked by anti-DQ antibody but not by anti-DR antibody in heterozygotes and homozygotes for HLA-DQ2. This may imply multiple epitopes within one sequence.

T cell epitopes in coeliac disease

15 Other investigators have characterized certain intestinal T cell clone epitopes. See, e.g., Vader et al., Gastroenterology 2002, 122:1729-37; Arentz-Hansen et al., Gastroenterology 2002, 123:803-809. These are examples of epitopes whose relevance is at best unclear because of the in vitro techniques used to clone T cells.

20 Intestinal versus peripheral blood clones

Intestinal: 1) intestinal biopsies, 2) T cell clones raised against peptic-tryptic digest of gluten, 3) all HLA-DQ2 restricted, 4) clones respond to gliadin deamidated by transglutaminase.

25 Peripheral blood: 1) T cell clones raised against gluten are HLA-DR, DQ and DP restricted. Result: Intestinal T cell clones can be exclusively used to map coeliac disease associated epitopes

30 GDA_9Wheat 307 aa Definition Alpha/Beta-Gliadin MM1 Precursor (Prolamin)
Accession P18573 – Genbank (which is incorporated herein by reference in its entirety)

Intestinal T cell clone epitopes

A definition of intestinal T cell clone epitopes can be found in, for example, Arentz-Hansen et al., J Exp Med. 2000, 191:603-12. Also disclosed therein are gliadin epitopes for intestinal T cell clones. Deamidated QLQPFPQPQLPY is an epitope, with a deamidated sequence of QLQPFPQPELPY. There is an HLA-DQ2 restriction. A homology search shows other bioactive α -gliadins include PQPQLPY singly or duplicated. A majority of T cell clones respond to either/or DQ2- α I: QLQPFPQPELPY DQ2- α II: PQPELPYPQPELPY

Dominant gliadin T cell epitopes

10 All deamidated by transglutaminase.

Peripheral blood day 6 after gluten challenge: A-gliadin 57-73:

QLQPFPQPELPYPQPQPS

Intestinal T cell clones: DQ2- α I: QLQPFPQPELPY DQ2- α II: PQPELPYPQPELPY

15 *Intestinal T-cell Clone Epitope Mapping*

α -Gliadins	A1	PFPQPQLPY
	A2	PQPQLPYQP
	A3	PYPQPQLPY
	Glia-20	PQQPYQPQPQ
20	Γ -Gliadins	G1 PQQSFPQQQ
		G2 IIPQQPAQ
		G3 FPQQPQQPYQPQQ
		G4 FSQPQQQFPQPQ
		G5 LQPQQPFPQQPQQPYQPQQPQ
25		Glu-21 QSEQSQQPFPQQF
		Glu-5 Q(IL)PQQPQQF
	Glutenin	Glt-156 PFSQQQQSPF
		Glt-17 PFSQQQQQ

Gluten exposure and induction of IFN γ -secreting A-Gliadin 57-73 QE65-specific T cells in peripheral blood

Untreated coeliac disease, followed by gluten free diet for 1, 2, or 8 weeks, followed by gluten exposure (3 days bread 200g/day), followed by gluten free diet

5 Result 1: Duration of gluten free diet and IFN γ ELISpot responses on day 0 and day 6 of gluten challenge: A-gliadin 57-73 QE65 (results expressed as IFN γ specific spots/million PPBMC)

Day 0: none (5), 1 week (1), 2 weeks (2), 8 weeks (1)

Day 6: none (0), 1 week (4), 2 weeks (28), 8 weeks (48)

10 Result 2: Duration of gluten free diet and IFN γ ELISpot responses on day 0 and day 6 of gluten challenge: tTG-gliadin (results expressed as IFN γ specific spots/million PPBMC)

Day 0: none (45), 1 week (62), 2 weeks (5), 8 weeks (5)

Day 6: none (0), 1 week (67), 2 weeks (40), 8 weeks (60)

15 Result 3: Duration of gluten free diet and IFN γ ELISpot responses on day 0 and day 6 of gluten challenge: A-gliadin 57-73 P65 (results expressed as IFN γ specific spots/million PPBMC)

Day 0: none (1), 1 week (2), 2 weeks (1), 8 weeks (1)

Day 6: none (0), 1 week (0), 2 weeks (0), 8 weeks (0)

20 Result 4: Duration of gluten free diet and IFN γ ELISpot responses on day 0 and day 6 of gluten challenge: PPD (results expressed as IFN γ specific spots/million PPBMC)

Day 0: none (90), 1 week (88), 2 weeks (210), 8 weeks (150)

Day 6: none (0), 1 week (100), 2 weeks (210), 8 weeks (100)

25 Result 5: Duration of gluten free diet and IFN γ ELISpot responses on day 0 and day 6 of gluten challenge: tTG (results expressed as IFN γ specific spots/million PPBMC)

Day 0: none (5), 1 week (4), 2 weeks (3), 8 weeks (2)

Day 6: none (0), 1 week (4), 2 weeks (1), 8 weeks (2)

30

Gluten challenge in HLA-DQ2 coeliac disease on long term gluten

Characterization of anti-gliadin T cell response was carried out in peripheral blood on day 6-8 after 3-day gluten challenge.

Result 1: PBMC Day 6 Long-term gluten free diet (preincubation with anti-HLA-DR and -DQ antibody) (expressed as % inhibition)

5 DR-: tTG-gliadin 100 mcg/ml (105), A-gliadin 57-73 QE65 50 mcg/ml (90),
PPD 5 mcg/ml (30)

DQ-: tTG-gliadin 100 mcg/ml (5), A-gliadin 57-73 QE65 50 mcg/ml (22),
PPD 5 mcg/ml (78).

Result 2: PBMC Day 6 Long-term gluten free diet (expressed as % CD8-depleted PBMC response)

B7 depletion: tTG-gliadin n=6 (7), A-gliadin 57-73 n=9 (6), PPD n=8 (62)

AE depletion: tTG-gliadin n=6 (120), A-gliadin 57-73 n=9 (80), PPD n=8 (110).

CD4 depletion: tTG-gliadin n=6 (10), A-gliadin 57-73 n=9 (9), PPD n=8 (10).

Therapeutic peptides include, but are not limited to

QLQPFPQPQLPYPQPQS (AG01)

QLQPFPQPQLPYPQPQP (AG02)

20 QLQPFPQPQLPYPQPQL (AG03)

QLQPFPQPQLPYLQPQP (AG04)

QLQPFPFPQLPYPQPQP (AG05)

QLQPFPQPQLPYSQPQP (AG06)

QLQPFLQPQLPYSQPQP (AG07)

25 QLQPFSPQPQLPYSQPQP (AG08)

QLQPFPQPQLSYSQPQP (AG09)

PQLPYPPQPQLPYPQPQP (AG10)

PQLPYPPQPQLPYPQPQL (AG11)

PQPQPFPLPQLPYPQPQS (AG12)

30 PQPQPFPPQPQLPYPQPQS (AG13)

PQPQPFPPQPQLPYPQYQP (AG14)

PQPQPFPPQPQLPYPQPPP (AG015)

Briefly after oral antigen challenge, specificities of peripheral blood T cells reflect those of intestinal T cell clones. In peripheral blood, epitopes of intestinal T cell clones are sub-optimal compared to A-gliadin 57-73 QE65, which is an optimal α -gliadin epitope.

Example 15

ELISpot assays were also carried out for mapping purposes as follows.

Fine-mapping the dominant DQ=8 associated epitope

	Sequence / sfc	tTG-treated sequence / sfc
10	VPQLQPQNPSQQQPQEJV / 76	RWPVPQLQPQNPSQQ / 60 WPVPQLQPQNPSQQQ / 90
	VPQLQPENPSQQQPQEJV / 3	PVPQLQPQNPSQQQP / 130
	VPQLQPRNPSQQQPQEJV / 76	VPQLQPQNPSQQQPQ / 140
15	VPQLQPQNPSSEQPQEJV / 100	PQLQPQNPSQQQPQE / 59
	VPQLQPQNPSQRQPQEJV / 1	QLQPQNPSQQQPQEJV / 95 LQPQNPSQQQPQEJV / 30 QPQNPSQQQPQEJV / 4
	VPQLQPQNPSQQQPEEQV / 71	
20	VPQLQPQNPSQQQPREQV / 27	DQ8 Gliadin Epitope GDA09 202Q / 6
	VPQLQPQNPSSEQPPEEQV / 81	GDA09 202E / 83
	VPQLQPENPSQQQPEEQV / 2	GDA09 202Q+tTG / 17
	VPQLQPENPSSEQPQEJV / 6	BI + tTG / 0
25	VPQLQPENPSSEQPPEEQV / 5	BI / 0

Fine-mapping dominant epitope (2)

Pool 33 (deamidated) / sfc

	A2b3 301 qqyp sgqg ffqp sqqn pqaq / 2
30	A2b5 301 qqyp sgqg ffqp fqqn pqaq / 1
	A3a1 301 qqyp sgqg ffqp sqqn pqaq / 0
	A3b1 301 qqyp ssqv sfqp sqln pqaq / 0

A3b2 301 qqyp ssqg sfqp sqqn pqaq / 2

A4a 301 eqyp sgqv sfqs sqqn pqaq / 28

A1b1 309 sfrp sqqn plaq gsvq pqql / 2

A1a1 309 sfrp sqqn pqaq gsvq pqql / 2

5

Example 16

Bioactivity of gliadin epitopes in IFN γ -ELISpot (25 mcg/ml, n=6) (expressed as % A-gliadin 57-73 QE65 response)

DQ2-AII: wild type (WT) (4), WT + tTG (52), Glu-substituted (52)

10 DQ2-AI: wild type (WT) (2), WT + tTG (22), Glu-substituted (28)

GDA09: wild type (WT) (1), WT + tTG (7), Glu-substituted (8)

A-G31-49: wild type (WT) (2), WT + tTG (3), Glu-substituted (0)

Dose response of A-Gliadin 57-73 QE65 (G01E) (n=8) (expressed as %G01E maximal response)

0.025 mcg/ml (1), 0.05 mcg/ml (8), 0.1 mcg/ml (10), 0.25 mcg/ml (22), 0.5 mcg/ml (38), 1 mcg/ml (43), 2.5 mcg/ml (52), 5 mcg/ml (70), 10 mcg/ml (81), 25 mcg/ml (95), 50 mcg/ml (90), 100 mcg/ml (85).

IFN γ ELISpot response to gliadin epitopes alone or mixed with A-gliadin

20 57-75 (G01E) (all 50 mcg/ml, tTG-gliadin 100 mcg/ml, PPD 5 mcg/ml; n=9) (expressed as % G01E response)

Alone: DQ2-A1 (20), DQ2-A2 (55), Omega G1 (50), tTG Gliadin (80), PPD (220), DQ2 binder (0)

G01E+: DQ2-A1 (90), DQ2-A2 (95), Omega G1 (100), tTG Gliadin (120),

25 PPD (280), DQ2 binder (80)

Effect of alanine and lysine substitution of A-gliadin 57-73 QE65 on IFN γ ELISpot responses in individual coeliac subjects (n=8)

Epitope sequence: QLQPFPQPELPYPQPQPS

30 Alanine substitution at positions 57-59 and 72-73 showed little to no decrease in % A-gliadin 57-73 QE65 response. Alanine substitution at positions 60-62 and 68-71 showed moderate decrease in % A-gliadin 57-73 QE65 response.

Alanine substitution at positions 63-67 showed most decrease in % A-gliadin 57-73 QE65 response.

Effect of lysine substitution of A-gliadin 57-73 QE65 on IFN γ ELISpot responses in individual coeliac subjects (n=8);

5 Epitope sequence: QLQPFPQPELPYPQPQSQ

Lysine substitution at positions 57-59 and 71-73 showed little to no decrease in % A-gliadin 57-73 QE65 response. Lysine substitution at positions 60-61 and 69-70 showed moderate decrease in % A-gliadin 57-73 QE65 response. Lysine substitution at positions 62-68 showed most decrease in % A-gliadin 57-73 QE65

10 response.

Example 17

Table 24 shows the results of analyses examining the 652 peptides with several patients challenged with wheat or rye.

15

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25 Each of the PCT publications, U.S. patents, other patents, journal references, and any other publications cited or referred to herein is incorporated herein by reference in their entirety.

Table 1. A-Gliadin protein sequence (based on amino acid sequencing)

5 VRVPVPQLQP QNPSQQQPQE QVPLVQQQQF PGQQQQFPPQ QPYQPQPFPF SQQPYLQLQP FPQPQLPYQP
 1 11 21 31 41 51 61
 PQSFPPQQPY PQPQPQYSQP QQPISQQQAQ QQQQQQQQQQ QQQILQQILQ QQLIPCMDVV LQQHNIAHAR
 71 81 91 101 111 121 131
 SQVLQQSTYQ LLQELCCQHL WQIPEQSQCQ AJHNVVHAI LHQQQKQQQQ PSSQVSFQQP LQQYP LGQGS
 141 151 161 171 181 191 201
 10 FRPSQQNPQA QGSVQPQQLP QFEEIRNLAL QTLPAMCNVY IAPYCTIAPF GIFTIN
 211 221 231 241 251 261

Table 2. Coeliac disease subjects studied

	Age Sex	Gluten free diet	HLA-DQ2	Bread challenge	Symptoms with bread
1	64 f	14 yr	Homozygote	3 days	Abdominal pain, lethargy, mouth ulcers, diarrhoea
2	57 m	1 yr	Heterozygote	10 days	Lethargy, nausea
3	35 f	7 yr	Heterozygote	3 days	Nausea
4	36 m	6 wk	Homozygote	3 days	Abdominal pain, mouth ulcers, diarrhoea
5	26 m	19 yr	Heterozygote	3 days	None
6	58 m	35 yr	Heterozygote	3 days	None
7	55 m	1 yr	Heterozygote	3 days	Diarrhoea
8	48 f	15 yr	Homozygote	3 days	Abdominal pain, diarrhoea

Aminoacid at position 65	Range	Mean
Glutamate	(100)	100%
Asparagine	(50-84)	70%
Aspartate	(50-94)	65%
Alanine	(44-76)	64%
Cysteine	(45-83)	62%
Serine	(45-75)	62%
Valine	(24-79)	56%
Threonine	(46-66)	55%
Glycine	(34-47)	40%
Leucine	(8-46)	33%
Glutamine	(16-21)	19%
Isoleucine	(3-25)	14%
Methionine	(3-32)	14%
Phenylalanine	(0-33)	12%
Histidine	(0-13)	8%
Tyrosine	(0-17)	8%
Tryptophan	(0-17)	8%
Lysine	(0-11)	4%
Proline	(0-4)	2%
Arginine	(0-2)	1%

Table 3

pt response G TG (3) 100 (100)	Peptide sequence	Corresponding residues in gliadin protein sequences (Accession no.)
7) 53 (44-67)	QLQPFPQPQLPYPQPQSQ	57-73 α-Gliadin (T. aestivum) Q41545
	QLQPFPQPQLPYPQPQSQ	57-73 α-Gliadin (T. aestivum) Q41545
	QLQPFPQPQLPYSQPQP	77-93 α/β-Gliadin precursor (Tricetum. aestivum) P02863
		76-92 α-Gliadin (T. aestivum) Q41528
		77-93 α-Gliadin storage protein (T. aestivum) Q41531
		57-73 α-Gliadin mature peptide (T. aestivum) Q41533
		77-93 α-Gliadin precursor (T. spelta) Q9ZP09
-20) 83 (61-113)	QLQPFPQPQLPYPQPQP	77-93 α/β-Gliadin A-II precursor (T. aestivum) P0472
-33) 83 (74-97)	QLQPFPQPQLPYPQPQP	77-93 α/β-Gliadin A-IV precursor (T. aestivum) P04724
		77-93 α/β-Gliadin MM1 precursor (T. aestivum) P18573
7) 109 (41-152)	PQLPYPQPQLPYPQPQP	84-100 α/β-Gliadin A-IV precursor (T. aestivum) P04724
	PQLPYPQPQLPYPQPQL	84-100 α/β-Gliadin MM1 precursor (T. aestivum) P18573
I) 3 (0-7)	QLQPFLQPQLPYSQPQP	77-93 α/β-Gliadin A-I precursor (T. aestivum) P04721
		77-93 α-Gliadin (T. aestivum) Q41509
I) 2 (0-7)	QLQPFSQPQLPYSQPQP	77-93 α-Gliadin storage protein (T. aestivum) Q41530
	PQPQPFPQPQLPYPOTQP	77-93 α/β-Gliadin A-III precursor (T. aestivum) P04723
-40) 24 (11-43)	PQPQPFPQPQLPYPQPQSQ	82-98 α/β-Gliadin A-V precursor (T. aestivum) P04725
-30) 19 (11-33)	PQPQPFPQPQLPYPQPQPP	82-98 α/β-Gliadin clone PW1215 precursor (T. aestivum) P04726
		82-98 α/β-Gliadin clone PW8142 precursor (T. aestivum) P04726
-30) 21 (11-33)	PQPQPFLQPQLPYPQPQSQ	79-95 α/β-Gliadin clone PW8142 precursor (T. aestivum) P04726
		79-95 α-Gliadin (T. aestivum) Q41529
		79-95 α/β-Gliadin precursor (T. aestivum) Q41546

Table 4

table 5. T cell epitopes described in coeliac disease

Source	Restriction	Frequency	Sequence*
imma-gliadin	DQ2	3/NS (iTCC)	QQLPQPEQPQQSFPEQERPF
pha-gliadin	DQ2	12/17 (iTCL)	QLQFPQPELPY
pha-gliadin	DQ2	11/17 (iTCL)	PQPELPYPQPELPY
pha-gliadin	DQ2	1/23 (bTCC)	LGQQQPFPPQQPYQPQPF
pha-gliadin	DQ8	3/NS (iTCC)	QQYPSGEGSFQPSQENPQ
utenin	DQ8	1/1 (iTCC)	GQQGYYPTSPQQSGQ
pha-gliadin	DQ2	11/12 in vivo	QLQFPQPELPYPQPQS

* not stated in original publication, iTCC intestinal T cell clone, iTCL intestinal polyclonal T cell line, bTCC peripheral blood cell clone

** peptides are the products of transglutaminase modifying wild type gluten peptides except the fourth and sixth peptides

table 6. Relative bioactivity of gliadin T cell epitopes in coeliac PBMC after gluten challenge

Sequence*	ELISpot response as % A-gliadin 57-73 Q865 (all 25mcg/ml)	Wild type	Wildtype+tTG	E-substituted
LPQPEQPQQSFPEQERPF	9 (3)	18 (7)	10 (5)	
QPFQPELPY	6 (2)	19 (1)	8 (3)	
PELPYPQPELPY	13 (6)	53 (8)	48 (9)	
YPSGEGSFQPSQENPQ	10 (3)	9 (3)	14 (8)	
QPFQPELPYPQPQS	18 (7)	87 (7)	100	
LPYFQPELPYPQPQS	14 (4)	80 (17)	69 (20)	

* sequence refers that of transglutaminase (tTG) modified peptide and the T cell epitope. Wild type is the unmodified gliadin peptide. Data from 4 subjects. Blank was 5 (1) %.

Table 7. Polymorphisms of A-gliadin 57-73

A. Sequences derived from Nordic autumn wheat strain Mjøelner

Alpha-gliadin protein (single letter code refers to Fig. 14 peptides)	Polymorphism
Q41545 A-gliadin (from sequenced protein) 57-73 (A)	QLQPFPQPQLPYPQPQS
Gli alpha 1,6: (EMBL: AJ133605 & AJ133602 58-74) (J)	QPQPFP P PQLPYPQTQP
Gli alpha 3,4,5: (EMBL: AJ133606, AJ133607, AJ133608 57-73) (I)	QLQPFPQPQL S QPQP
Gli alpha 7: (EMBL: AJ133604 57-73) (E)	QLQPFP R PQLPYPQPQP
Gli alpha 8, 9, 11: (EMBL:) (F)	QLQPFPQPQLPYSQPQP
Gli alpha 10: (EMBL: AJ133610 57-73) (D)	QLQPFPQPQLPYLQPQS

5 B. SWISSPROT and TREMBL scan (10.12.99) for gliadins containing the sequence: XXXXXXXPQLPYXXXX

Wheat (Triticum aestivum unless stated) gliadin accession number	Polymorphism
Q41545 A-gliadin (from sequenced protein) 57-73 (A)	QLQPFPQPQLPYPQPQS
SWISSPROT:	
GDA0_WHEAT P02863 77-93 (F)	QLQPFPQPQLPYSQPQP
GDA1_WHEAT P04721 77-93 (G)	QLQPFLQPQLPYSQPQP
GDA2_WHEAT P04722 77-93 (B)	QLQPFPQPQLPYPQPQP
GDA3_WHEAT P04723 77-93 (O)	POPOPFPQPQLPYPQPQP
GDA4_WHEAT P04724 77-93 (C)	QLQPFPQPQLPYPQPQL
GDA4_WHEAT P04724 84-100 (K)	POLPYPQPQLPYPQPQP
GDA5_WHEAT P04725 82-98 (N)	POPOPFEPQLPYPQPQS
GDA6_WHEAT P04726 82-98 (P)	POPOPFPQPQLPYPQPPE
GDA7_WHEAT P04727 79-95 (M)	POPOPFLPQLPYPQPQS
GDA9_WHEAT P18573 77-93 (C)	QLQPFPQPQLPYPQPQL
GDA9_WHEAT P18573 84-100 (L)	POLPYPQPQLPYPQPQL
GDA9_WHEAT P18573 91-107 (K)	POLPYPQPQLPYPQPQP
TREMBL	
Q41509 ALPHA-GLIADIN 77-93 (G)	QLQPFLQPQLPYSQPQP
Q41528 ALPHA-GLIADIN 76-92 (F)	QLQPFPQPQLPYSQPQP
Q41529 ALPHA-GLIADIN 79-95 (M)	POPOPFLPQLPYPQPQS
Q41530 ALPHA-GLIADIN 77-93 (H)	QLQPF S QPQLPYSQPQP
Q41531 ALPHA-GLIADIN 77-93 (F)	QLQPFPQPQLPYSQPQP
Q41533 ALPHA-GLIADIN 57-73 (F)	QLQPFPQPQLPYSQPQP
Q41546 ALPHA/BETA-GLIADIN 79-95 (M)	POQPFLPQLPYPQPQS
Q41632 ALPHA/BETA-TYPE GLIADIN. Triticum urartu 82-98 (P)	POPOPFPQPQLPYPQPPE
Q9ZP09 ALPHA-GLIADIN Triticum spelta 77-93 (F)	QLQPFPQPQLPYSQPQP

Table 8. Bioactivity of substituted variants of A-gliadin 57-73 QE65 (Subst) compared to unmodified A-gliadin 57-73 QE65 (G) (mean 100%, 95% CI 97-104) and blank (no peptide, bl) (mean 7.1%, 95% CI: 5.7-8.5)

Subst	%	P vs G	Subst	%	P vs G	Subst	%	P vs G	Subst	%	P vs G	P vs bl
Super-agonists												
Y61	129	<0.0001	Y63	71	<0.001	H62	47	<0.0001	N66	24	<0.0001	
	1					G69	47	<0.0001	R64	24	<0.0001	
Y70	129	0.0006	S69	70	<0.0001	N63	47	<0.0001	K63	23	<0.0001	
Agonists												
W70	119	0.017	F63	70	0.008	M68	46	<0.0001	H66	23	<0.0001	
K57	118	0.02	P70	69	<0.0001	D68	46	<0.0001	H67	22	<0.0001	
Y59	117	0.04	T62	69	<0.0001	V69	46	<0.0001	L64	22	<0.0001	
A57	116	0.046	L61	69	<0.0001	G63	45	<0.0001	S66	22	<0.0001	
S70	116	0.045	S61	69	<0.0001	V64	45	<0.0001	F67	21	<0.0001	
K58	114	0.08	T61	69	<0.0001	E61	45	<0.0001	W66	21	<0.0001	
W59	110	0.21	T63	69	<0.0001	A69	43	<0.0001	G64	21	<0.0001	
A73	109	0.24	M66	68	<0.0001	R62	42	<0.0001	G65	21	<0.0001	
I59	108	0.37	T69	67	<0.0001	G68	42	<0.0001	D64	21	<0.0001	
G59	108	0.34	K60	66	<0.0001	A64	42	<0.0001	I65	21	<0.0001	
A58	108	0.35	S62	66	<0.0001	C65	42	<0.0001	M64	20	<0.0001	<0.0001
W60	105	0.62	M61	66	<0.0001	N67	41	<0.0001	G67	19	<0.0001	<0.0001
A59	104	0.61	P61	65	<0.0001	W63	41	<0.0001	T65	19	<0.0001	0.003
K72	104	0.65	M62	64	<0.0001	F69	41	<0.0001	A66	19	<0.0001	<0.0001
S59	103	0.76	Q61	64	<0.0001	N68	40	<0.0001	I64	19	<0.0001	0.0003
K73	102	0.8	G61	64	<0.0001	V66	40	<0.0001	R63	19	<0.0001	<0.0001
A70	102	0.81	A63	64	<0.0001	H69	40	<0.0001	W67	19	<0.0001	<0.0001
Y60	101	0.96	L62	60	<0.0001	M69	40	<0.0001	K68	18	<0.0001	<0.0001
A72	100	0.94	I68	60	<0.0001	R69	40	<0.0001	H64	18	<0.0001	<0.0001
S63	98	0.67	S67	59	<0.0001	W69	40	<0.0001	W64	18	<0.0001	0.0001
K59	96	0.46	N61	59	<0.0001	Q69	39	<0.0001	Q65	18	<0.0001	0.0002
I60	96	0.5	I69	59	<0.0001	L67	38	<0.0001	F64	16	<0.0001	0.0008
G70	95	0.41	V61	58	<0.0001	K69	38	<0.0001	L65	16	<0.0001	0.0022
D65	95	0.44	D61	58	<0.0001	K62	38	<0.0001	N64	16	<0.0001	<0.0001
E70	93	0.27	E60	57	<0.0001	E67	37	<0.0001	F65	16	<0.0001	0.12
I63	92	0.19	A61	57	<0.0001	L69	37	<0.0001	Q67	15	<0.0001	0.0012
S60	92	0.23	Q62	56	<0.0001	S64	36	<0.0001	M65	14	<0.0001	0.015
P59	88	0.08	F68	56	<0.0001	G62	36	<0.0001	D66	14	<0.0001	0.013
M63	87	0.03	N65	56	<0.0001	E69	36	<0.0001	R67	14	<0.0001	0.002
K71	85	0.047	A62	56	<0.0001	E68	36	<0.0001	Non-agonists			
V62	84	0.04	A68	53	<0.0001	V67	35	<0.0001	P63	13	<0.0001	0.012
I70	84	0.04	P66	53	<0.0001	D62	35	<0.0001	E64	12	<0.0001	0.053
I61	83	0.01	R61	53	<0.0001	R68	34	<0.0001	W65	11	<0.0001	0.24
V68	82	0.0045	S68	53	<0.0001	Q66	34	<0.0001	Q64	11	<0.0001	0.15
E59	81	0.01	Y63	52	<0.0001	A67	33	<0.0001	G66	11	<0.0001	0.07
Partial agonists												
W61	79	0.002	E63	51	<0.0001	F66	31	<0.0001	Y67	10	<0.0001	0.13
A60	78	0.002	T64	51	<0.0001	E62	31	<0.0001	E66	10	<0.0001	0.17
Y62	78	0.006	T67	51	<0.0001	D69	31	<0.0001	K66	10	<0.0001	0.21
G60	77	0.003	Y69	50	<0.0001	D67	30	<0.0001	R66	10	<0.0001	0.23
A71	77	0.003	D63	50	<0.0001	M67	29	<0.0001	K67	10	<0.0001	0.11
W62	76	0.0009	A65	49	<0.0001	Y66	28	<0.0001	P65	8	<0.0001	0.57
Q60	76	0.001	K61	49	<0.0001	I67	28	<0.0001	K64	8	<0.0001	0.82
L63	74	0.0002	I66	49	<0.0001	H65	26	<0.0001	K65	8	<0.0001	0.63
I62	74	0.0005	T68	48	<0.0001	P68	26	<0.0001	Y65	7	<0.0001	0.9
K70	74	0.001	S65	48	<0.0001	Y64	25	<0.0001				
H61	72	<0.0001	L68	48	<0.0001	EK65	25	<0.0001				
W68	72	<0.0001	Q68	48	<0.0001	T66	25	<0.0001				

5 Table 9. Antagonism of A-gliadin 57-73 QE65 interferon gamma ELISPOT response by substituted variants of A-gliadin 57-73 QE65 (Subst) (P is significance level in unpaired t-test). Agonist activity (% agonist) of peptides compared to A-gliadin 57-73 QE65 is also shown.

Subst	% Inhibit.	P	% agonist.	Subst	% Inhibit.	P	% agonist.
Antagonists			65R	13	0.18	11	
65T	28	0.004	19	65M	13	0.16	14
67M	27	0.0052	29	68P	13	0.16	26
64W	26	0.007	18	63R	13	0.19	19
67W	25	0.0088	19	66G	12	0.19	11
Potential antagonists				65Q	12	0.2	18
67I	24	0.013	10	65Y	12	0.22	7
67Y	24	0.013	21	66S	12	0.22	22
64G	21	0.03	21	67F	11	0.25	21
64D	21	0.029	16	66R	10	0.29	10
65L	20	0.046	26	67K	10	0.29	10
66N	20	0.037	24	64F	10	0.29	16
65H	20	0.038	16	65F	9	0.41	16
64N	19	0.05	16	63P	8	0.42	13
64Y	19	0.06	25	65EK	8	0.39	25
66Y	19	0.048	28	64Q	7	0.49	11
64E	19	0.049	12	64I	5	0.6	21
67A	18	0.058	30	68K	5	0.56	19
67H	18	0.052	22	67Q	5	0.61	18
Non-antagonists				65G	5	0.62	15
65V	17	0.07	23	64M	4	0.7	20
65I	17	0.086	21	66H	4	0.66	23
66T	17	0.069	25	66E	3	0.76	10
65W	15	0.11	11	66D	1	0.9	14
67R	15	0.13	14	63K	1	0.88	23
65P	15	0.13	8	64H	1	0.93	18
65K	15	0.11	8	66K	0	0.98	10
66W	15	0.12	21	64K	-2	0.88	8
67G	14	0.14	19	64L	-11	0.26	22
66A	14	0.14	19				

Table 10. Inhibition of A-gliadin 57-73 QE65 interferon gamma ELISPOT response by peptides known to bind HLA-DQ2 (P is significance level in unpaired t-test).

Peptide	% Inhibit.	P
TP	31	<0.0001
HLA1a	0	0.95

Table 11. Antagonism of A-gliadin 57-73 QE65 interferon gamma ELISpot response by naturally occurring polymorphisms of A-gliadin 57-73 QE65 (P is significance level in unpaired t-test).

A-gliadin 57-73 QE65 polymorphism		% Inhibit.	P
P04725 82-98 QE90	<u>PQPOPFPPELPYPQPQS</u>	19	0.009
Q41509 77-93 QE85	<u>QLQPFLQPELPYSQPQP</u>	11	0.15
Gli α 1,6 58-74 QE66	<u>QPQPFPPELPYPQTQP</u>	11	0.11
P04723 77-93 QE85	<u>POPOPFPPELPYPQTQP</u>	10	0.14
Gli α 3-5 57-73 QE65	<u>QLQPFPQPELSYSQPQP</u>	7	0.34
P02863 77-93 QE85	<u>QLQPFPQPELPYSQPQP</u>	6	0.35
Q41509 77-93 QE85	<u>QLQPFLQPELPYSQPQP</u>	6	0.41
P04727 79-95 QE65	<u>POPOPFLPELPYPQPQS</u>	6	0.39
P04726 82-98 QE90	<u>POPOPFPPELPYPQPQQP</u>	5	0.43

Table 12. Prolamin homologues of A-gliadin 57-73 (excluding alpha/beta-gliadins)

Prolamin	Accession number	Sequence	% Bioactivity*
Wheat: α -gliadin	A-gliadin (57-73)	QLQPFPQPQLPYPQPQS	100 (0)
Wheat: ω -gliadin	AAG17702 (141-157)	PQ.....F.....QSE	32 (6.4)
Barley: C-hordein	Q40055 (166-182)	...QPFPL.....F.....Q	2.3 (2.0)
Wheat γ -gliadin	P21292 (96-112)	...QTFPQ.....F.....QPQ	2.1 (4.2)
Rye: secalin	Q43639 (335-351)	...QPSPQ.....F.....Q	1.6 (1.4)
Barley: γ -hordein	P80198 (52-68)	...QPFPQ.....HQHQFP	-1.0 (1.8)
Wheat: LMW glutenin	P16315 (67-83)	LQ...QPIL.....FS...Q...Q	-0.9 (1.0)
Wheat: HMW glutenin	P08489 (718-734)	HGYYPTS.....SGQQQRP	6.4 (4.0)
Wheat γ -gliadin	P04730 (120-136)	...QCCQQL.....L...QQSRYQ	0.7 (0.9)
Wheat: LMW glutenin	P10386 (183-199)	...QCCQQL.....L...QQSRYE	-0.7 (0.5)
Wheat: LMW glutenin	O49958 (214-230)	...QCCRQL.....L...EQSRYD	-1.1 (0.3)
Barley: B1-hordein	P06470 (176-192)	...QCCQQL.....L...EQFRHE	1.8 (1.4)
Barley: B-hordein	Q40026 (176-192)	...QCCQQL.....ISEQFRHE	0.5 (0.9)

*Bioactivity is expressed as 100x(spot forming cells with peptide 25mcg/ml plus tTG 8mcg/ml minus blank)/(spot forming cells with A-gliadin 57-73 25mcg/ml plus tTG 8mcg/ml minus blank) (mean (SEM), n=5).

Peptides were preincubated with tTG for 2h 37°C. Note, Q is deamidated in A-gliadin 57-73 by tTG.

Table 13. Clinical details of coeliac subjects.

	HLA-DQ	HLA-DQA1 alleles	HLA-DQB1 alleles	Duodenal histology	Gluten free	EMA on gluten (on GFD)
C01	2, 6	102/6, 501	201, 602	SVA	1 yr	+ (-)
C02	2, 2	501	201	SVA	1 yr	+ (-)
C03	2, 5	101/4/5, 501	201, 501	PVA	1 yr	+ (-)
C04	2, 5	101/4-5, 501	201, 501	SVA	7 yr	+ (-)
C05	2, 2	201, 501	201, 202	SVA	4 mo	+ (ND)
C06	2, 2	201, 501	201, 202	SVA	2 yr	+ (-)
C07	2, 8	301-3, 501	201, 302	SVA	1 yr	+ (-)
C08	2, 8	301-3, 501	201, 302/8	SVA	11 yr	ND (-)
C09	2, 8	301-3, 501	201, 302	SVA	29 yr	+ (-)
C10	2, 8	201, 301-3	202, 302	IEL	1 yr	+ (-)
C11	6, 8	102/6, 301-3	602/15, 302/8	IEL	9 mo	- (ND)
C12	8, 7	301-3, 505	302, 301/9-10	SVA	2 yr	- (-)
C13	8, 8	301	302	SVA	1 yr	+ (+)

SVA subtotal villous atrophy, PVA partial villous atrophy, IEL increased intra-epithelial atrophy, GFD gluten-free diet, ND not done.

Table 14. HLA-DQ2+ Coeliac (C01-6) and healthy control (H01-10) IFN γ ELISpot responses to control peptides (20 μ g/ml) and gliadin (500 μ g/ml) before and after gluten challenge (sfc/million PBMC minus response to PBS alone)

Peptide	Healthy Day 0	Healthy Day 6	Coeliac Day 0	Coeliac Day 6
P04722 77-93	0 (-4 to 17)	0 (-5 to 9)	-2 (-3 to 0)	27 (0-100)*
P04722 77-93 + tTG	0 (-5 to 4)	0 (-9 to 3)	0 (-4 to 11)	141 (8 to 290)**
P04722 77-93 QE85	0 (-5 to 5)	0 (-3 to 4)	0 (-6 to 14)	133 (10 to 297)*
P02863 77-93	0 (-4 to 13)	2 (-3 to 5)	-2 (-3 to 2)	8 (-2 to 42)**
P02863 77-93 + tTG	-1 (-5 to 4)	-1 (-4 to 11)	1 (-4 to 6)	65 (8-164)**
P02863 77-93 QE85	0 (-4 to 13)	0 (-4 to 14)	-1 (-4 to 6)	42 (-2 to 176)*
Gliadin chymotrypsin	2 (-5 to 20)	18 (0 to 185)*	20 (11 to 145)	92 (50 to 154)
Gliadin chymotrypsin + tTG	0 (-1 to 28)	16 (-9 to 171)*	55 (29 to 248)	269 (206 to 384)**
Chymotrypsin	0 (-4 to 5)	1 (-4 to 11)	-2 (-5 to 5)	1 (-4 to 8)
Chymotrypsin + tTG	0 (-5 to 8)	6 (0 to 29)	-2 (-3 to 11)	2 (-3 to 18)*
Gliadin pepsin	4 (-4 to 28)	29 (0 to 189)***	44 (10 to 221)	176 (54 to 265)**
Gliadin pepsin + tTG	2 (-3 to 80)	27 (-4 to 241)***	61 (8 to 172)	280 (207 to 406)**
Pepsin	0 (-4 to 10)	0 (-3 to 12)	0 (-2 to 3)	2 (-2 to 8)
Pepsin + tTG	0 (-3 to 8)	0 (-5 to 9)	1 (-6 to 3)	0 (-3 to 14)
PBS alone	4 (0 to 6)	2 (0 to 6)	4 (1 to 12)	4 (0 to 4)
PBS + tTG	3 (0 to 8)	3 (0 to 11)	4 (2 to 10)	4 (2 to 11)

5 Day 6 vs. Day 0: *P<0.05 **P<0.02, ***P<0.01 by one-tailed Wilcoxon Matched-Pairs Signed-Ranks test

Table 15. Effect of deamidation by tTG to gliadin (0.5 mg/ml) and A-gliadin 57-73 homologues on IFN γ ELISpot responses in HLA-DQ2+ coeliac (C01-6) and healthy control subjects (H01-10) (median ratio tTG: no tTG pretreatment, 5 range)

Peptide	Healthy Day 6	Celiac Day 0	Celiac Day 6
Gliadin chymotrypsin	0.94 (0.4-9.0)	2.1 (0.8-6.8)*	3.2 (1.8-4.2)**
Gliadin pepsin	1.4 (0.5-1.4)	1.4 (0.8-4.0)*	1.9 (1.1-4.4)**
P04722 77-93 Q85			6.5 (2.3-12)**
P04722 77-93 E85			0.7 (0.6-1.1)
P02863 77-93 Q85			7.5 (3.9-19.9)**
P02863 77-93 E85			1.0 (0.8-1.2)

tTG>no tTG: *P<0.05 **P<0.02, ***P<0.01 by one-tailed Wilcoxon Matched-Pairs Signed-Ranks test

Table 16. Healthy subjects: IFN γ ELISpot Responses (>10 sfc/million PBMC and >4 x buffer only) to tTG-treated gliadin peptide Pools on Day 6 of gluten challenge (sfc/million PBMC) (*italic*: response also present on Day 0):

Group 1 – HLA-DQ2 (DQA1*0501-5, DQB1*0201)

5 Group 2 – HLA-DQ8 (DQA1*0301, DQB1*0302) and absent or “incomplete” DQ2 (only DQA1*0501-5 or DQB1*0201)

Table 17: tTG-deamidated gliadin peptide pools showing significant increase in IFN gamma responses between Day 0 and Day 6 of gluten challenge in HLA-DQ2 coeliac subjects C01-6 (Day 6 -Day 0 response, and ratio of responses to 5 tTG-deamidated pool and same pool without tTG treatment)

Pool	IFNg ELISpot		Pool	IFNg ELISpot	
	(Median sfc/million)	tTG: no tTG (Median)		(Median sfc/million)	tTG: no tTG (Median)
9	59***	1.0	49	46***	1.4
10	116**	1.7	50	50***	4.6
11	24***	2.5	51	40***	1.7
12	133***	1.1	52	30***	3.1
13	26**	2.1	53	27**	1.4
42	30**	1.2	76	17***	1.1
43	32***	1.3	79	20***	0.9
44	24***	1.5	80	83***	1
45	10***	1.1	81	141***	1.1
46	12***	2.1	82	22***	1.5
48	17***	1.4	83	16**	1.8

Day 6 vs. Day 0 **P<0.02, ***P<0.01 by one-tailed Wilcoxon Matched-Pairs Signed-Ranks test

Table 18. Coeliac subjects: IFN γ ELISpot Responses >10 sfc/million PBMC and >4 x buffer only to tTG-treated Pepset Pools on Day 6 of gluten challenge (sfc/million PBMC) (*italic*: response also present on Day 0):

Group 1 – HLA-DQ2 (DQA1*0501-5, DQB1*0201/2),

5 Group 2 – HLA-DQ2/8 (DQA1*0501-5, *0301, and DQB1*0201/2, *0302), and

Group 3 – HLA-DQ8 (DQA1*0301, DQB1*0302) and absent or “incomplete”

DQ2 (only DQA1*0501-5 or DQB1*0201/2)

Subject	Group 1:						Group 2:			Group 3			
	C01	C02	C03	C04	C05	C06	C07	C08	C09	C10	C11	C12	C13
HLA-DQ	2, 6	2, 2	2, 5	2, 5	2, 2	2, 2	2, 8	2, 8	2, 8	2, 8	6, 8	7, 8	8, 8
Pool 1	23
2	223	.	.	.
3	155	.	.	.
4	11	41	.	.	.
5	22	.	.	.
6	18	.	.	.	21	.	20	17
7	353
8	11	64	20	480	.	.	13	.	.
9	93	127	.	92	25	.	32	460	.	.	18	.	.
10	175	491	58	200	48	.	84	787
11	32	118	.	33	14	.	26	27	.	12	.	.	.
12	204	379	54	225	61	.	129	587	.	12	.	.	.
13	93	142	.	29	18	.	60	.	.	.	11	.	.
14	.	45	.	21	.	.	17
15	18	30	38	43
16	37
17
18
19	11
20	11	215	51	167	11
21
22	.	21	12	.	.	.
23	.	18	.	21	12	.	.	.
24	.	15	10	.	.	12	.	.	.
25	.	15	12	.	.	.
26	.	18	13	.	.	12	.	.	.
27	.	15	12	.	.	.
28	11	.
29
30	11	11
31	.	70	11
32	.	18	20
33	11	.	.	10	.	.	14	11	.	40	11	.	.
34	11
35
36
37	.	.	.	23	14
38	.	24	.	19	.	.	20
39	.	49	.	15	.	.	.	11
40	14

Table 19. Deamidated peptides with mean bioactivity > 10% of P04722 E85 (20 µg/ml) in HLA-DQ2 coeliac subjects C01-5

Rank	No.	Sequence	Mean (SEM)	Rank	No.	Sequence	Mean (SEM)
*2	89	PQLPYPQQLPYPQQLPQQLPYP	94 (18)	37	413	SKQPKQQPPQPKQQPKQSQFQ	18 (4)
*3	91	PQPFPQQLPYPQQLPYPQQP	89 (12)	38	380	QPQQPKQQPFPQPKQQPKLPFP	18 (6)
*4	74	MQLQPPQQLPYPQQLPYPQY	88 (14)	39	618	PQQSFSYQQQPFQQFYPQQ	18 (7)
*5	90	PQLPYPQQLPYPQQLPQPF	87 (16)	*40	78	LQLQPFPRPQLPYPQQLPFP	17 (8)
*6	76	LQLQPPQQLPYPQQLPQPF	85 (15)	41	390	QQTYPQRPQQPFFQFQQPQQ	17 (9)
6	626	PQQPQCPQQPQQPQQPQQPFPW	72 (23)	42	548	QQYFQQPQQTFFHQPKQQQPP	16 (10)
7	627	QPFPQCPQQPFPWQPKQQFFQ	66 (30)	43	409	QPQQPFQQLQQPKQQPQPKQ	16 (2)
*8	631	FPQCPQCPQFQPKQLPFPQS	61 (12)	44	382	QQCPQCPQCPQFQFQCPQQ	16 (6)
9	636	PQQPQCPFPQCPQCPFPVQPK	51 (10)	45	629	PPFQTPQSFPLQPKQCPFPQQ	16 (5)
*10	73	LQLQPPQCPQQLPYPQQLPY	49 (11)	46	643	PLQCPQCPQCPQCPQCPQCP	16 (6)
11	412	SQQPQCPQCPQCPQCPQCPQ	34 (19)	47	389	QQFPFQTPQCPQCPFQCPQ	16 (6)
12	343	QQPQCPFPQCPQCPQCPFPQ	34 (11)	48	350	QQFPQCPQCPFQCPQCPQCP	15 (8)
*13	68	LQLQPPQCPQQLPVLQPKPF	33 (10)	49	65	PPFSQCPYCPQCPFQCPQCP	15 (5)
*14	66	LQLQCPFPQCPQQLPYSQCPF	32 (7)	50	349	QQFPQCPQCPFQCPQCPQCP	15 (9)
*15	96	PQFPFPQQLPYPQPKQCPQQ	28 (6)	51	610	FWQQQPKLPPQKQSEQQPPF3	15 (11)
16	393	QLPFPQCPQCPFPQCPQCPQ	27 (8)	*52	81	PPQCPFPQCPQCPFPQCPQ	15 (5)
17	355	QAFPQCPQCPFPQCPQCPQCP	27 (15)	*53	73	MQLQPFQCPQCPFPQCPQCP	14 (5)
*18	67	LQLQPPQCPQQLPYSQCPQCP	26 (6)	54	368	QQCPQCPQCPQCPFPQCPQ	14 (7)
19	335	QQQQPFPQCPQCPQCPFPQCP	25 (11)	*55	82	PPQCPFPQCPCPFPQCPQCP	14 (3)
*20	95	PQFPFLPQLPYPQCPQCPFPQ	24 (6)	*56	83	LQLQPPQCPQCPFPQCPQCP	14 (4)
21	396	TQCPQCPFPQCPQCPFPQCP	23 (9)	57	624	FTQCPQCPFPQCPQCPFPQ	14 (6)
22	609	SCISCLERPWQQQPQLPPQGS	23 (18)	58	407	QPPQCPFPQCPQCPFPQCP	14 (5)
23	385	-QQFPFPQCPQCPFPQCPQ	23 (7)	59	337	QQCPQCPFPQCPFPQCPQ	13 (4)
24	375	PQQPFPQCPQCPFPQCPQCP	23 (10)	60	634	PQQLQCPFPQCPQCPFPQCP	13 (3)
25	406	QCPQCPFPQCPQCPFPQCP	22 (8)	61	388	QQFPFPQCPQCPFPQCPQCP	13 (3)
26	625	PQCPQCPFPQCPQCPFPQCP	22 (9)	62	641	FPELQCPFPQCPQCPFPQCP	13 (7)
27	378	QQCPQCPFPQCPQCPFPQCP	22 (10)	63	399	QQCPQCPFPQCPQCPFPQCP	13 (5)
28	371	PQQCPQCPFPQCPQCPFPQCP	22 (10)	64	387	QQTCPQCPFPQCPQCPFPQCP	13 (4)
29	642	PQQCPQCPFPQCPQCPFPQCP	20 (8)	65	628	PPFWQCPQCPFPQCPFPQCP	12 (4)
30	635	PLQPQCPFPQCPQCPFPQCP	19 (5)	*66	88	PPFPFPQCPFPQCPFPQCP	12 (3)
*31	93	PQFPFPQQLPYPQCPFPQCP	19 (5)	67	408	QPQCPFPQCPFPQCPFPQCP	12 (5)
32	377	PQQCPQCPFPQCPQCPFPQCP	19 (9)	*68	77	LQLQPPQCPQCPFPQCPFPQCP	11 (4)
33	411	LOQPQCPFPQCPQCPFPQCP	19 (4)	69	370	PQQCPFPQCPFPQCPFPQCP	11 (5)
34	415	SQQPQCPFPQCPQCPFPQCP	18 (5)	*70	79	LQLQPPQCPFPQCPFPQCP	11 (5)
*35	94	PQFPFPQQLPYPQCPFPQCP	18 (5)	71	379	QQCPQCPFPQCPFPQCPFPQCP	11 (5)
36	329	PSQVQVWQCPFPQCPFPQCP	18 (4)	72	397	PPQCPQCPFPQCPFPQCP	11 (3)

* Indicates homologue of A-gliadin 57-73 with the core sequence PQLP(Y/F)

Table 20. Peptides >10% as bioactive as P04722 QE65 grouped by structure.

Rank	Peptide no. (Pool) Gliadin-subtype	Sequence	IFNg ELISpot response compared to P04722 77-93 QE85: mean (SEM)
Group 1: Homologues of A-gliadin 57-73			
	P04722 77-93	QLQPFPPQQLPYPQPQP	
1	89 (12) α	PQL...Y.....LPY	94 (18)
2	91 (12) α	PQPFPPQQL...Y.....	89 (12)
3	74 (10) α	M.....LPY	88 (14)
4	90 (12) α	PQL...Y.....PFRP	87 (16)
5	76 (10) α	L.....PFR	85 (19)
8	631 (81) ω	FPQQPQ...F...QS	61 (12)
10	73 (10) α	L.....LPY	49 (11)
13	68 (9) α	L.....L.....PFR	33 (10)
14	66 (9) α	L.....S.....PFR	32 (7)
18	67 (9) α	L.....S.....QFR	26 (6)
20	95 (13) α	PQPFL.....FPPQQ	24 (6)
31	93 (12) α	PQPFP.....PFRPQQ	19 (5)
35	94 (12) α	PQPFP.....PPFSPQQ	18 (3)
40	78 (10) α	L.....R.....PFR	17 (8)
52	81 (11) α	PQPQFP.....T...PFPP	15 (5)
53	75 (10) α	MQLQPFPPQQPQF.....	14 (5)
55	82 (11) α	PQPQPFPPQQPQF.....	14 (3)
56	80 (10) α	LQLQPFPPQQPQF.....	14 (4)
66	88 (11) α	PQPFP.....S.....PFRPQQ	12 (3)
68	77 (10) α	LQLQPFPPQQPQF.....	11 (4)
70	79 (10) α	LQLQPFPPQQPQF.....	11 (5)
Group 2: Homologues of peptide 626			
		QQFPFPQPPQQFP	
6	626(80) ω	PQQPQQP.....W	72 (23)
7	627(80) ωWQPQQFPQ	66 (30)
9	636(81) ω	PQQP.....I...VQPO	51 (10)
11	412(53) γ	SQQP.....Q.....PQQ	34 (19)
33	411(53) γ	LQQP.....Q.....PQQ	19 (4)
36	329(42) γ	PSGQVQWPQ.....	18 (4)
41	390(50) γ	QOTYPORP.....T.....QQ	17 (9)
59	337(43) γ	Q.....CQQPQRTI	13 (4)
61	388(50) γ	QQPYPPQQP.....T.....QQ	13 (3)
Group 3: Homologues of peptide 355			
		FPQQPQQTFPHQPQQQFP	
17	355(46) γ	QA.....Q	27 (15)
42	348(45) γ	QQT.....	16 (10)
48	350(45) γ	QQI.....A.....	15 (8)
50	349(45) γ	QQL.....	15 (9)
Group 4: Homologues of Peptide 396			
		QQPFPOQPQQPFP	
21	396(51) γ	TQQP.....QTQ	23 (9)
27	378(49) γ	QQP.....QPOQ	22 (10)
28	371(48) γ	PQQQFIQP.....TY	22 (10)
29	642(82) ω	PQQP.....L.....QQP	20 (8)
30	635(81) ω	PLQP.....QPO	19 (5)
44	382(49) γQTQQPQQ	16 (6)
45	629(81) ω	PFPQT...S...L.....QQ	16 (5)
46	643(82) ω	PLQP.....QQP	16 (6)
60	634(81) ω	PQQL.....L.....QQP	13 (3)
64	387(50) γT.....L.....QQPOQOPF	13 (4)
62	641(82) ω	FPEL.....I.....LQP	13 (7)

Group 5: Homologues of Peptide 343 (overlap Groups 2 and 4)			
		QQPFPQPQQPQLPFPQ	
12	343(44) γ	QQP.....Q	34 (11)
16	393(51) γ	QLPFPQQP.....	27 (8)
19	335(43) γ	QQ.....Q.....PQ	25 (11)
23	385(50) γQPQQ	23 (7)
24	375(48) γ	P.....Q.....PQQ	23 (10)
25	406(52) γ	QP.....L.....Q.....PQ	22 (8)
32	377(49) γ	P.....Q.....Q.....QPQ	19 (9)
34	415(53) γ	SQQP.....QS.....	18 (5)
37	413(53) γ	SKQP.....QS.....	18 (4)
38	380(49) γ	QPQQP.....	18 (6)
43	409(53) γ	QP.....L.....Q.....L.....PQ	16 (2)
47	389(50) γT.....Q.....QPQQ	16 (6)
58	407(52) γ	QP.....S.....Q.....PQ	14 (5)
63	399(51) γT.....Q.....LQQP	13 (5)
67	408(52) γ	QP.....SK.....Q.....PQ	12 (5)
71	379(49) γ	QQP.....Q.....Q.....P	11 (5)
72	397(51) γ	PQQP.....T.....Q.....	11 (3)
Group 6: Peptide 625			
		PIQPQQPFPQQP	
26	625(80) ωQQPQQPFP	22 (9)
57	624(80) ω	FTQPQQPT.....	14 (6)
65	628(80) ω	PF...W.....TQQSFPLQ	12 (4)
Group 7: Peptide 618			
39	618(79) ω	PQQSFSYQQQPFPQQPYPPQQ	18 (7)

Table 21. Bioactivity of individual tTG-deamidated Pools 1-3 peptides in Subject C12:

No.	Sequence	%	No.	Sequence	%
8	<u>AVRWPVPOLQPNPQQQPO</u>	100	23	<u>LOPQNPNPSQQQPOEQVPLMQQ</u>	26
		85			18
5	MVRVTVVPQ.....	14	EQVPLVQQ	
		82			18
6	AVRVSVVPQ.....	15	H.....EQVPLVQQ	
		77			18
3	MVRVPVPQ.....H.....	17	KQVPLVQQ	
		67			13
1	AVRFPVPQ.....L.....	16	D.....EQVPLVQQ	
		59			8
2	MVRVPVPQ.....	13	EQVPLVQQ	
		49			5
9	AVRVPVPQ.....L.....	22	K.....EQVPLVQQ	
		49			3
7	AVRVPVPQ.....	18	L.....EQVPLVQE	
		33			3
10	MVRVPVPQ.....L.....	19	L.....EQVPLVQE	
4	MVRVPMPQ.....D.....	15	20	P.....P.....GQVPLVQQ	0
12	AVRVPVPQ.....K.....	8	21	P.....P.....RQVPLVQQ	0
11	AVRVPVPQP.....P.....	0			

Core sequence of epitope is underlined. Predicted deamidated sequence is: LQPENPSQEQQPE

Table 22: Phylogenetic groupings of wheat (*Triticum aestivum*) gliadins

Alpha/beta-gliadins (n=61)			
Ala1	AAA96525, EEWTA, P02863	A1b13	B22364, P04271
Ala2	CAB76963	A2a1	AAB23109, CAA35238, P18573, S10015
Ala3	AAA96276	A2a2	CAB76964
Ala4	CAA26384, S07923	A2b1	P04724, T06500, AAA348282
Ala5	AAA34280	A2b2	D22364
Ala6	P04728	A2b3	P04722, T06498, AAA34276
Alb1	CAB76962	A2b4	C22364
Alb2	CAB76961	A2b5	CAB76956
Alb3	BAA12318	A3a1	AAA34277, CAA26383, P04726, S07361
Alb4	CAB76960	A3a2	I307187B, A27319, S13333
Alb5	CAB76958	A3b1	AAA96522
Alb6	CAB76959	A3b2i	AAA34279, P04727,
Alb7	CAB76955	A3b2ii	CAA26385, S07924
Alb8	AAA96524	A3b3	A22364, AAA34278, AAB23108, C61218, P04725
Alb9	CAA10257	A4a	P04723, AAA34283, T06504
Alb10	AAA96523, T06282	A4b	E22364
Alb11	AAA17741, S52124	A4c	CAB76957
Alb12	AAA34281	A4d	CAB76954
Gamma-gliadins (n=47)			
Gl1a	P08079, AAA34288, P30094, CAC11079, AAD30556, CAC11057, CAC11065, CAC11056	Gl5a	AAK84774, AAK84772
Gl1b	CAC11089, CAC11064, CAC11080, CAC11078, AAD30440	Gl5b	AAK84773
Gl1c	CAC11087	Gl5c	AAK84776
Gl1d	CAC11088	Gl6a	JA0153, P21292, AAA34272, I507333A
Gl1e	CAC11055	Gl6b	AAK84777
Gl2a	JS0402, P08453, AAA34289	Gl6c	1802407A, AAK84775, AAK84780
Gl2b	AAK42989, AAK84779, AAK84779	Gl7	AAB31090
Gl3a	AAK84778	Gl8a	AAA34287, P04730, S07398
Gl3b	CAB75404	Gl8b	I209306A
Gl3c	BAA11251	Gl9a	P04729
Gl4	EEWTG, P06659, AAA34274	Gl9b	AAA34286
Omega-gliadins (n=3)			
O1a	AAG17702		
O1b	P02865		
O1c	A59156		

Table 23. Synthetic peptides spanning all known wheat gliadin 12mers

Protein	Position*	Sequence	No.	Protein	Position*	Sequence	No.
POOL 1							
A1A1	20	AVRF PVPQ LQPQ NPSQ QLPQ	1	G12A 33	QQQL VPQL QQPL SQQP QQTF	331	
A1A2	20	MVRV PVPQ LQPQ NPSQ QQPQ	2	G13A 33	QQQP FPQP HQPF SQQP QQTF	332	
A1B1	20	MVRV PVPQ LQPQ NPSQ QHPQ	3	G14 33	QQQP FLQP HQPF SQQP QQIF	333	
A1B2	20	MVRV PMPQ LQPQ DPSQ QQPQ	4	G15A 33	QQQQ PFPQ PQQP FSQQ PQQI	334	
A1B7	20	MVRV TVPQ LQPQ NPSQ QQPQ	5	G15B 33	QQQQ PFPQ PQQP QQPQ PQQ	335	
A1B8	20	AVRV SVPQ LQPQ NPSQ QQPQ	6	G15C 33	QQQP FRQP QQPQ YQQP QTTF	336	
A1B8	20	AVRV PVPQ LQPQ NPSQ QQPQ	7	G16A 33	QQQP FPQP QQPQ CQQP QRTI	337	
A1B10	20	AVRW PVPQ LQPQ NPSQ QQPQ	8	G16C 42	QQQP FPQP QQPQ CEQP QRTI	338	
POOL 2							
A2B3	20	AVRV PVPQ LQLQ NPSQ QQPQ	9	G11A 42	HQPF SQQP QQTF PQPQ QTFF	339	
A2B5	20	MVRV PVPQ LQLQ NPSQ QQPQ	10	G12A 42	QQPL SQQP QQTF PQPQ QTFF	340	
A3A1	20	AVRV PVPQ PQPQ NPSQ PQPQ	11	G14 42	HQPF SQQP QQIF PQPQ QTFF	341	
A3B1	20	AVRV PVPQ LQPQ NPSQ QQPQ	12	G15A 42	QQPF SQQP QQIF PQPQ QTFF	342	
A1A1	28	LQPQ NPSQ LQPQ EQVP LVQQ	13	G15B 42	QQPF QPFP PQQQ PQLP FPQQ	343	
A1A2	28	LQPQ NPSQ LQPQ EQVP LVQQ	14	G15C 42	QQPF YQQP QTTF PQPQ QTCP	344	
A1B1	28	LQPQ NPSQ QHPQ EQVP LVQQ	15	G16A 42	QQPF CQQP QRTI PQPH QTFF	345	
A1B2	28	LQPQ DPSQ QQPQ EQVP LVQQ	16	G16B 42	QQPF CQQP QRTI PQPH QTFF	346	
POOL 3							
A2B1	28	LQPQ NPSQ QQPQ KVVP LVQQ	17	G16C 42	QQPF CEQP QRTI PQPH QTFF	347	
A2B3	28	LQLQ NPSQ QQPQ EQVP LVQE	18	G11A 50	QTFP PQPQ QTFF HQPQ QQFP	348	
A2B5	28	LQLQ NPSQ QQPQ EQVP LVQE	19	G14 50	QQIF PQPQ QTFF HQPQ QQFP	349	
A3A1	28	PQPQ NPSQ PQPQ GQVP LVQQ	20	G15A 50	QQIF PQPQ QTFF HQPQ QAFF	350	
A3A2	28	PQPQ NPSQ PQPQ RQVP LVQQ	21	G16A 50	QRTI PQPH QTFF HQPQ QTFF	351	
A3B1	28	LQPK NPSQ QQPQ EQVP LVQQ	22	G15A 58	QTFP HQPQ QAFF PQQQ TFPH	352	
A4A	28	LQPQ NPSQ QQPQ EQVP LMQQ	23	G16A 58	QTFH HQPQ QTFF PQQQ TYPH	353	
A1A1	36	QLPQ EQVP LVQQ QQFL GQQQ	24	G16C 58	QTFH HQPQ QTFF PQEQ TYPH	354	
POOL 4							
A1B1	36	QHQP EQVP LVQQ QQFL GQQQ	25	G15A 66	QAFF PQQQ TFPH PQQQ QFPQ	355	
A1B2	36	QQPQ EQVP LVQQ QQFL GQQQ	26	G15C 66	QHTF PQPQ QTCP HQPQ QQFP	356	
A1B12	36	QQPQ EQVP LVQQ QQFL GQQQ	27	G16A 66	QTFP PQQQ TYPH PQQQ QFPQ	357	
A2A1	36	QQPQ EQVP LVQQ QQFP GQQQ	28	G16C 66	QTFP QPEQ TYPH PQQQ QFPQ	358	
A2B1	36	QQPQ KVVP LVQQ QQFP GQQQ	29	G11A 73	QTFP HQPQ QQFP PQQQ PQQQ	359	
A2B3	36	QQPQ EQVP LVQQ QQFP GQQQ	30	G12A 73	QTFP HQPQ QOVP PQQQ PQQQ	360	
A3A1	36	PQPQ GQVP LVQQ QQFP GQQQ	31	G13A 73	QTFP HQPQ QOFS PQQQ PQQQ	361	
A3A2	36	PQPQ RQVP LVQQ QQFP GQQQ	32	G15C 73	QTCP HQPQ QQFP PQQQ PQQQ	362	
POOL 5							
A4A	36	QQPQ EQVP LMQQ QQFP PGQQ	33	G16A 73	QTYP HQPQ QQFP QTQQ PQQP	363	
A1A1	44	LVQQ QQFL GQQQ PFPQ QOFP	34	G11A 81	QOFP PQPQ PQQQ FLQP QOFP	364	
A1B1	44	LVQQ QQFL GQQQ SFPP QOFP	35	G12A 81	QOVP PQQQ PQQP FLQP QOFP	365	
A1B12	44	LVQQ QQFL GQQQ PPPP QOFP	36	G13A 81	QQFS PQQQ PQQQ FIOP QOFP	366	
A2A1	44	LVQQ QQFP GQQQ PPPP QOFP	37	G14 81	QQFP PQQQ PQQQ FLQP RQFP	367	
A2B3	44	LVQE QQFP GQQQ PPPP QOFP	38	G15A 81	QQFP PQQQ PQQP PQQQ PQQQ	368	
A3A1	44	LVQQ QQFP GQQQ QFPF QOFP	39	G16A 81	QQFP PQQQ PQQP FPQP QOFP	369	
A4A	44	LMQQ QQFP PGQQ EQFP PQQP	40	G11A 89	PQQQ FLQP QOFP PQQP QOFP	370	
POOL 6							
A4D	44	LMQQ QQFP PGQQ ERFP PQQP	41	G13A 89	PQQQ FIQP QOFP PQQP QOFP	371	
A1A1	53	GQQQ PFPF QOFP PQQP PFPF	42	G13B 89	PQQQ FIQP QOFP QTYP QRFP	372	
A1A3	53	GQQQ PFPF QOFP PQQP PFPF	43	G14 89	PQQQ FIQP RQFP PQQP QOFP	373	
A1B1	53	GQQQ SFPP QOFP PQQP PFPF	44	G15A 89	PQQQ FIQP PQQQ PQFP QOFP	374	
A2B1	53	GQQQ SFPP QOFP PQQP PFPF	45	G15C 89	PQQQ FIQP PQFP QOFP PQQQ	375	
A3A1	53	GQQQ QFPF QOFP PQQP PFPF	46	G16A 89	PQQP FPQP QOFP PQQP QLFP	376	
A4A	53	GQQE QFPF QOFP PHQQ PFPF	47	POOL 49			
A4D	53	GQQE RFPF QOFP PHQQ PFPF	48	G15A 97	PQQQ FPQP QOFP QOFP PQQQ	377	
A1A1	61	QQPY PQPQ PFPS QLPY LQLQ	49	G15A 105	QQPQ QOFP QOFP QOFP PQQQ	378	
A1A3	61	QQPY PQPQ PFPS LPLY LQLQ	50	G15A 113	QQPQ QOFP QOFP PQQQ PQQP FPQP	379	
A1B1	61	QQPY PQPQ PFPS QOFP LQLQ	51	G15A 121	QPQP PQQP FPQP QOFP LPPF	380	
A2B1	61	QQPY PQQQ PFPS QOFP MQLQ	52	G12A 126	QPQP PQQP QOFP PQQP PQQQ	381	
A4A	61	QQPY PHQQ PFPS QOFP PQQP	53	G13A 126	QPQP PQQP QOFP PQQP PQQQ	382	
A1A1	69	PFPS QLPY LQLQ PFPQ PQLP	54	G14 126	RQFP PQQP QOFP PQQP PQQQ	383	
A1B1	69	PFPS QOFP LQLQ PFPQ PQLP	55	POOL 50			
A1B10	69	PFPS QOFP LQLQ PFSQ PQLP	56	G15A 126	QPQP PQQP QOFP PQFP QOFP	384	
POOL 7							
A1A1	61	QQPY PQPQ PFPS QLPY LQLQ	57	G15C 126	QPQP PQQP QOFP PQFP QOFP	385	
A1A3	61	QQPY PQPQ PFPS LPLY LQLQ	58	G16A 126	QPQP PQQP QLFP PQQP QOFP	386	
A1B1	61	QQPY PQPQ PFPS QOFP LQLQ	59	G11A 134	QPQP PQQP QOFP PQFP QOFP	387	
A2B1	61	QQPY PQQQ PFPS QOFP MQLQ	60	G12A 134	QPQP PQQP QOFP PQFP PQQQ	388	
A4A	61	QQPY PHQQ PFPS QOFP PQQP	61	G13A 134	QPQP PQFP QOFP PQQQ PQFP	389	
A1A1	69	PFPS QOFP LQLQ PFPQ PQLP					
A1B11	69	PFPS QOFP LQLQ PFLQ PQLP					
A1B12	69	PFPS QOFP LQLQ PFLQ PQLP					
A2A1	69	PFPS QOFP LQLQ PFPQ PQLP					
A2B1	69	PFPS QOFP MQLQ PFPQ PQLP					
A2B2	69	PFPS QOFP MQLQ PFPQ PQLP					

A2B4	69 PFPS QQPY LQLQ PFPQ PQPF	62 GISC 134 QAQL PFPQ QPQQ PLPQ PQQP	392
A2B5	69 PFPS QQPY LQLQ PFPR PQLP	63 POOL 51	
A4A	69 PFPS QQPY PQPQ PEPP QLPY	64 G16A 134 QLPF POOP QOPF POPO QPOO	393
POOL 9		64 G12A 142 QPQQ PFPQ QPQQ PFPQ TQQP	394
A4B	69 PFPS QQPY PFPQ PFPQ PQPF	65 G12A 150 QPQQ PFPQ TQQP QPQQ PFPQ	395
A1A1	77 LQLQ PFPQ PQLP YSQP QPFR	66 G12A 158 TQQP QPQQ PFPQ QPQQ PQTQ	396
A1A4	77 LQLQ PFPQ PQLP YSQP QQFR	67 G12A 166 PQQP QQPQ PQTQ QPQQ PFPQ	397
A1B1	77 LQLQ PFPQ PQLP YLQP QPFR	68 G11A 170 QOPF PQTQ QPQQ LFPQ SQQP	398
A1B4	77 LQLQ PFPQ PQLS YSQP QPFR	69 G12A 170 QOPF PQTQ QPQQ PFPQ LQQP	399
A1B10	77 LQLQ PFSQ PQLP YSQP QPFR	70 G13A 170 QOPF PQTQ QPQQ PFPQ SQQP	400
A1B11	77 LQLQ PFLQ PQLP YSQP QPFR	71 POOL 52	
A1B12	77 LQLQ PFLQ PQLP PQFP PPQL PYSQ	72 G14 170 QOPF PQTQ QPQQ PFPQ SKQP	401
POOL 10		72 G15A 170 QOPF PFPQ QPQQ PFPQ LQQP	402
A2A1	77 LQLQ PFPQ PQLP YPQP QLPY	73 GISC 170 QOPL POPO QPQQ PFPQ SQQP	403
A2B1	77 MQLQ PFPQ PQLP YPQP QLPY	74 G16A 170 QOPF PFPQ QPQQ PFPQ SQQP	404
A2B2	77 MQLQ PFPQ PQLP YPQP YPQP	75 G11A 178 QOPQ LFPPQ SQQP QQQF SQQP	405
A2B3	77 LQLQ PFPQ PQLP YPQP QPFR	76 G12A 178 QOPQ PFPQ LQQP QOPF PFPQ	406
A2B4	77 LQLQ PFPQ PQPQ PPQL PYQP	77 G13A 178 QOPQ PFPQ SQQP QOPF PFPQ	407
A2B5	77 LQLQ PFPF PQLP YPQP QPFR	78 G14 178 QPQQ PFPQ SKQP QOPF PFPQ	408
A3B1	77 LQLQ PFPQ PQPP LPQL PYQP	79 POOL 53	
A3B3	77 LQLQ PFPQ PQPP PQLP PYQP	80 G15A 178 QPQQ PFPQ LQQP QQPL PQPQ	409
POOL 11		80 G11A 186 SQQP QQFQ SQQP QOPF QPQQ	410
A4A	77 PQPQ PFPQ QLPY PQTQ PFPF	81 G12A 186 LQQP QOPF POPO QQLP QPQQ	411
A4B	77 PQPQ PFPQ PQPP PPQL PYQP	82 G13A 186 SQQP QOPF PFPQ QQFQ SQQP	412
A1A1	85 PQLP YSQP QPFR PQQP YPQP	83 G14 186 SKQP QOPF PFPQ QPQQ SFQP	413
A1A6	85 PQLP YSQP QPFR PQQP YPQP	84 G15A 186 LQQP QQPL PFPQ QPQQ PFPQ	414
A1B1	85 PQLP YLQP QPFR PQQP YPQP	85 G15C 186 SQQP QOPF PFPQ QPQQ SFQP	415
A1B4	85 PQLS YSQP QPFR PQQP YPQP	86 G11A 194 SQQP QOPF PFPQ PQQS FPQQ	416
A1B6	85 PQLS YSQP QPFR PQQL YPQP	87 POOL 54	
A1B12	85 PQPF PPQL PYSQ PFPF RPQQ	88 G12A 194 PQPQ QQLP QPQQ PQQS PFPQ	417
POOL 12		88 G13A 194 PQPQ QQFP QPQQ PQQS PFPQ	418
A2A1	85 PQLP YPQP QLPY PQPQ LPYP	89 G14 194 PQPQ PFPQ SFQP QQPS LQQP	419
A2B1	85 PQLP YPQP QLPY PQPQ PRFP	90 G15A 194 PFPQ PFPQ PFPQ QQQP LQQP	420
A2B2	85 PQPQ PPQL PYQP PQLP YPQP	91 G15C 194 PFPQ PFPQ SFQP QQQP LQQP	421
A2B3	85 PQLP YPQP QPFR PQQP YPQP	92 G11A 202 PQPQ PQQS FPQQ QOPF IQPS	422
A2B4	85 PQPQ PPQL PYQP PFPF RPQQ	93 G12A 202 QPQQ PQQS FPQQ QRPF IQPS	423
A3A1	85 PQPF PPQL PYQP PPQQ SPQQ	94 G13A 202 QPQQ PQQS FPQQ QPSL IQQS	424
POOL 13		POOL 55	
A3B1	85 PQPF LPQL PYQP PQSF PPQQ	95 G11A 210 FPQQ QOPF IQPS LQQQ VNPC	425
A3B3	85 PQPF PPQL PYQP PQSF PPQQ	96 G12A 210 FPQQ QOPF IQPS LQQQ LNPC	426
A4A	85 QLPY PQTQ PFPQ QQPY PQPQ	97 G13A 210 FPQQ QPSL IQQS LQQQ LNPC	427
A4B	85 PQPF PPQL PYQP TOPF PPQQ	98 G15A 210 FPQQ QOPF IQPS LQQQ MNPC	428
A2A1	106 LPYP QOPF FRPQ QPYP QSQP	99 G16A 210 FPQQ QQPA IQQS LQQQ MNPC	429
A2B1	106 LPYP QOPF FRPQ QSYP QOPP	100 G11A 218 IQPS LQQQ VNPC KNFL LQQC	430
A3A1	106 LPYP QPPP FSQP QPYP QOPP	101 G12A 218 IQPS LQQQ LNPC KNFL LQQS	431
A3B1	106 LPQL PYQP PQSF PPQQ PYQP	102 G13A 218 IQQS LQQQ LNPC KNFL LQQC	432
POOL 14		POOL 56	
A4A	106 PPQL PYQP TOPF PPQQ PYQP	103 G15A 218 IQPY LQQQ MNPC KNYL LQQC	433
A1A1	112 QPFR PQQP YPQP QPQY SQPQ	104 G16A 218 IQSF LQQQ MNPC KNFL LQQC	434
A1B6	112 QPFR PQQL YPQP QPQY SQPQ	105 G11A 226 VNPC KNFL LQQC KPVS LVSS	435
A2A1	112 QPFR PQQP YPQS QPQY SQPQ	106 G12A 226 LNPC KNFL LQQS KPAS LVSS	436
A2B2	112 QPFR PQQS YPQP QPQY SQPQ	107 G13A 226 LNPC KNFL LQQC KPVS LVSS	437
A3A1	112 PPFS PQQP YPQP QPQY SQPQ	108 G15A 226 MNPC KNYL LQQC NPVS LVSS	438
A3B1	112 QSFP PQQP YPQQ RPKY LQQP	109 G16A 226 MNPC KNFL LQQC NHVS LVSS	439
A3B2	112 QSFP PQQP YPQQ RPML LQQP	110 G11A 234 LQQC KPVS LVSS LWSM IWQP	440
POOL 15		POOL 57	
A3B3	112 QSFP PQQP YPQQ QPQY LQQP	111 G12A 234 LQQS KPAS LVSS LWSI IWQP	441
A4A	112 QPFP PQQP YPQP QPQY SQPQ	112 G13A 234 LQQC KPVS LVSS LWSM ILPR	442
A1A1	112 YPQP QPQY SQPQ QPIS QQQQ	113 G15A 234 LQQC NPVS LVSS LWSM ILPR	443
A1B3	120 YPQP QPQY SQPQ EPIS QQQQ	114 G16A 234 LQQC NHVS LVSS LWSI ILPR	444
A2A1	120 YPQS QPQY SQPQ QPIS QQQQ	115 G11A 242 LVSS LWSM IWQP SDCQ VMRQ	445
A3A1	120 YPQP QPQY SQPQ QPIS QQQA	116 G12A 242 LVSS LWSI IWQP SDCQ VMRQ	446
A3B1	120 YPQQ RPKY LQQP QPIS QQQA	117 G13A 242 LVSS LWSM ILPR SDCQ VMRQ	447
A3B2	120 YPQQ RPML LQQP QPIS QQQA	118 G14 242 LVSS LWSI ILPP SDCQ VMRQ	448
POOL 16		POOL 58	
A3B3	120 YPQQ QPQY LQQP QPIS QQQA	119 G15A 242 LVSS LWSM ILPR SDCQ VMRQ	449
A1A1	128 SQPQ QPIS QQQQ QQQQ QQQQ	120 G15C 242 LVSS LWSM ILPR SDCQ VMQQ	450
A1B3	128 SQPQ EPIS QQQQ QQQQ QQQQ	121 G16A 242 LVSS LWSI ILPR SDCQ VMQQ	451
A3A1	128 PQPQ QPIS QQQA QQQQ QQQQ	122 G11A 250 IWQP SDCQ VMRQ QCCQ QLAQ	452
A1A1	138 QQQQ QQQQ QQQQ QQQQ ILQQ	123 G13A 250 ILPR SDCQ VMRQ QCCQ QLAQ	453
A1A6	138 QQQQ QQQQ QQQQ QQQQ QEQQ ILQQ	124 G14 250 ILPR SDCQ VMRQ QCCQ QLAQ	454
A1B11	138 QQQQ QQQQ QQQQ QQQQ QQQQ IIQQ	125 G15A 250 ILPR SDCQ VMRQ QCCQ QLAQ	455

A2AI 138 QQQQ QQQQ QKQ QQQQ QQQI	126 G15C 250 ILPR SDCQ VMQQ QCCQ QLAQ	456
POOL 17	POOL 59	
A4B 139 AQQQ QQQQ QQQQ QQQQ TLQQ	127 G11A 258 VMRQ QCCQ QLAQ IPQQ LQCA	457
A1AI 146 QQQQ QQQQ ILQQ ILQQ QLIP	128 G15A 258 VMRQ QCCQ QLAQ IPQQ LQCA	458
A1A6 146 QQQQ QEQQ ILQQ ILQQ QLIP	129 G15C 258 VMQQ QCCQ QLAQ IPQQ LQCA	459
A1B6 146 QQQQ QEQQ ILQQ MLQQ QLIP	130 G16A 258 VMQQ QCCQ QLAQ IPQQ LQCA	460
A1B10 146 QQQQ QEQQ ILQQ ILQQ QLTP	131 G11A 266 QLAQ IPQQ LQCA AIHT IIHS	461
A1B11 146 QQQQ QQQQ IIHQ ILQQ QLIP	132 G11B 266 QLAQ IPQQ LQCA AIHT VVHS	462
A2AI 146 QQQQ QQQQ QQQI LQQI LQQQ	133 G12A 266 QLAQ IPQQ LQCA AIHS VVHS	463
A3A2 146 QQQQ QQQQ ILPQ ILQQ QLIP	134 G13A 266 QLAQ IPQQ LQCA AIHS IVHS	464
POOL 18	POOL 60	
A4A 146 QQQQ QQQQ TLQQ ILQQ QLIP	135 G14A 266 QLAR IPQQ LQCA AIHG IVHS	465
A1AI 163 ILQQ ILQQ QLIP CMDV VLQQ	136 G15C 266 QLAQ IPQQ LQCA AIHS VVHS	466
A1B6 163 ILQQ MLQQ QLIP CMDV VLQQ	137 G16A 266 QLAQ IPQQ LQCA AIHS VAHS	467
A1B10 163 ILQQ ILQQ QLTP CMDV VLQQ	138 G11A 274 LQCA AIHT IIHS IIHQ QEQQ	468
A2B1 163 ILQQ QLIP CRDV VLQQ	139 G11B 274 LQCA AIHT VVHS IIHQ QEQQ	469
A3A2 163 ILPQ ILQQ QLIP CRDV VLQQ	140 G12A 274 LQCA AIHS VVHS IIHQ QQQQ	470
A4A 163 TLQQ ILQQ QLIP CRDV VLQQ	141 POOL 61	
A1AI 171 QLIP CMDV VLQQ HNIA HGRS	142 G13A 274 LQCA AIHS IVHS IIHQ QEQQ	471
POOL 19	143 G14 274 LQCA AIHS VVHS IIHQ QEQQ	472
A1A3 171 QLIP CMDV VLQQ HNKA HGRS	144 G16A 274 LQCA AIHS VAHS IIHQ QEQQ	473
A1B2 171 QLIP CMDV VLQQ HNLA HGRS	145 G11A 282 IIHS IIHQ QEQQ EQQQ GMHI	474
A1B7 171 QLIP CMDV VLQQ HNIV HGRS	146 G11B 282 VVHS IIHQ QEQQ QGMH ILLP	475
A1B10 171 QLTP CMDV VLQQ HNIA RGRS	147 G12A 282 VVHS IIHQ QQQQ QQQQ QGID	476
A1B11 171 QLIP CMDV VLQQ HNIV HGKS	148 G13A 282 IVHS IIHQ QEQQ EQRQ GVQI	477
A2A1 171 QLIP CRDV VLQQ HSIA YGSS	149 POOL 62.	
A2B1 171 QLIP CRDV VLQQ HSIA HGSS	150 G14 282 VVHS IIHQ QEQQ EQLQ GVQI	479
A2B3 171 QLIP CRDV VLQQ HNIA HGSS	151 G15A 282 IVHS IIHQ QEQQ QQQQ QQQQ	480
POOL 20	152 G16C 282 VVHS IVMQ QEQQ QGIP ILRP	481
A3A1 171 QLIP CRDV VLQQ HNIA HARS	153 G16A 282 VAHS IIHQ QEQQ QGVP ILRP	482
A3B1 171 QLIP CRDV VLQQ HNIA HASS	154 G11A 290 QEQQ EQQQ GMHI LLPL YQQQ	483
A1AI 179 VLQQ HNIA HGRS QVLQ QSTY	155 G12A 290 QEQQ QQQQ QGID IFPL LSQH	484
A1A3 179 VLQQ HNKA HGRS QVLQ QSTY	156 G12B 290 QEQQ QQQQ QGMH IFPL LSQQ	485
A1B2 179 VLQQ HNLA HGRS QVLQ QSTY	157 G13A 290 QEQQ EQRQ GVQI LVPL SQQQ	486
A1B7 179 VLQQ HNIV HGRS QVLQ QSTY	158 G14 290 QEQQ EQLQ GVQI LVPL SQQQ	487
A1B10 179 VLQQ HNIA RGRS QVLQ QSTY	159 G15A 290 QEQQ QQQQ QQQQ QQQQ IQIM	488
A1B11 179 VLQQ HNIV HGKS QVLQ QSTY	160 G15C 290 QEQQ QGIP ILRP LFQL VQGQ	489
POOL 21	161 G16A 290 QEQQ QGVP ILRP LFQL AQGL	490
A2A1 179 VLQQ HSIA YGSS QVLQ QSTY	162 G11A 305 GMHI LLPL YQQQ QVGQ GTLV	491
A2B1 179 VLQQ HSIA HGSS QVLQ QSTY	163 G12A 305 GIDJ FLPL SQHE QVGQ GSLV	492
A2B3 179 VLQQ HNIA HGSS QVLQ ESTY	164 G12B 305 GMHI FLPL SQQQ QVGQ GSLV	493
A3A1 179 VLQQ HNIA HARS QVLQ QSTY	165 POOL 64	
A3B1 179 VLQQ HNIA HASS QVLQ QSTY	166 G13A 305 GVQI LVPL SQQQ QVGQ GTLV	495
A4A 179 VLQQ HNIA HASS QVLQ QSSY	167 G14 305 GVQI LVPL SQQQ QVGQ GLIV	496
A1AI 187 HGRS QVLQ QSTY QLLQ ELCC	168 G15A 305 GQIP MRPL FQLV QQQG IIQP	497
A1A3 187 HGRS QVLQ QSTY QLLQ ELCC	169 G16A 305 GVPJ LRPL FQLA QGLG IIQP	498
A2A1 187 YGSS QVLQ QSTY QLVQ QLCC	170 G11A 313 YQQQ QVGQ GTLV QGQG IIQP	499
A2B1 187 HGSS QVLQ QSTY QLVQ QFCC	171 G12A 313 SQHE QVGQ GSLV QGQG IIQP	500
A2B3 187 HGSS QVLQ ESTY QLVQ QLCC	172 G12B 313 SQQQ QVGQ GSLV QGQG IIQP	501
A3A1 187 HARS QVLQ QSTY QPLQ QLCC	173 POOL 65	
A3B1 187 HASS QVLQ QSTY QLLQ QLCC	174 G13A 313 SQQQ QVGQ GTLV QGQG IIQP	502
A4A 187 HASS QVLQ QSSY QQLQ QLCC	175 G14 313 SQQQ QVGQ GLIV QGQG IIQP	503
POOL 23	176 G11A 321 GTLV QGQG IIQP QQPA QLEA	504
A1AI 195 QSTY QLLQ ELCC QHLW QIPE	177 G12A 321 GSLV QGQG IIQP QQPA QLEA	505
A1A3 195 QSTY QLLR ELCC QHLW QIPE	178 G15A 321 FQLV QGQG IIQP QQPA QLEV	506
A1B8 195 QSTY QLLR ELCC QHLW QIPE	179 G16A 321 FQLA QGLG IIQP QQPA QLEG	507
A2A1 195 QSTY QLVQ QLCC QQLW QIPE	180 G13A 329 IIQP QQPA QLEA IRSI VLQT	508
A2B1 195 QSTY QLVQ QFCC QQLW QIPE	181 POOL 66	
A3A1 195 QSTY QPLQ QLCC QQLW QIPE	182 G13C 329 IIQP QQPA QLEV IRSI VLQT	509
A3B1 195 QSTY QLLQ QLCC QQLL QIPE	183 G15C 329 IIQP QQPA QYEV IRSI VLRT	510
A4A 195 QSSY QQLQ QLCC QQLF QIPE	184 G16A 329 IIQP QQPA QLEG IRSI VLKT	511
POOL 24	185 G11A 337 QLEA IRSI VLQT LPTM CNVY	512
A1AI 203 ELCC QHLW QIPE QSQC QAIH	186 G12A 337 QLEA IRSI VLQT LPSM CNVY	513
A1B6 203 ELCC QHLW QILE QSQC QAIH	187 G13A 337 QLEV IRSI VLQT LATM CNVY	514
A1B10 203 ELCC QHLW QIPE KLQC QAIH	188 G15A 337 QLEV IRSI VLGT LPTM CNVY	515
A2A1 203 QLCC QQLW QIPE QSRC QAIH	POOL 67	516
A2B1 203 QFCC QQLW QIPE QSRC QAIH		517
A3B1 203 QLCC QQLL QIPE QSRC QAIH		518
POOL 25		

A3B3 203 GLCC QQLL QIPE QSQC QAIH	189 G15C 337 QEYV IRSL VLRT LPNM CNVY	519
A4A 203 QLCC QQLF QIPE QSRC QAIH	190 G16A 337 QLEG IRSL VLKT LPTM CNVY	520
A1A1 211 QIPE QSQC QAIH NVVH AII	191 G11A 345 VLQT LPTM CNVY VPPE CSII	521
A1B3 211 QIPE QSQC QAIQ NVVH AII	192 G12A 345 VLQT LPSM CNVY VPPE CSIM	522
A1B6 211 QILE QSQC QAIH NVVH AII	193 G13A 345 VLQT LATM CNVY VPPE CSTI	523
A1B9 211 QIPE QSQC QAIH KVHH AII	194 G14A 345 VLGT LPTM CNVF VPPE CSTT	524
A1B10 211 QIPB KLQC QAIH NVVH AII	195 G15C 345 VLRT LPNM CNVY VRPD CSTI	525
A2A1 211 QIPE QSRC QAIH NVVH AII	196 G16A 345 VLKT LPTM CNVY VPPD CSTI	526
POOL 26	POOL 68	
A3B3 211 QIPE QSQC QAIH NVVH AII	197 G11A 353 CNVY VPPE CSII KAPF SSVV	527
A4A 211 QIPE QSRC QAIH NVVH AII	198 G12A 353 CNVY VPPE CSIM RAPP ASIV	528
A1A1 219 QAIH NVVH AII HQQQ KQQQ	199 G13A 353 CNVY VPPI CSTI RAPP ASIV	529
A1A6 219 QAIH NVVH AII HQQQ KQQQ	200 G14A 353 CNVY VPPE CSTT KAPF ASIV	530
A1B3 219 QAIQ NVVH AII HQQQ KQQQ	201 G15C 353 CNVY VRPD CSTI NAPP ASIV	531
A1B9 219 QAIH KVHH AII HQQQ KQQQ	202 G16A 353 CNVY VPPD CSTI NVPY ANID	532
A1B13 219 QAIH NVVH AII HQQQ KQQQ	203 G11A 361 CSII KAPF SSVV AGIG GQ	533
A2B3 219 QAIH NVVH AII HQQQ KVHH	204 G12A 361 CSIM RAPP ASIV AGIG GQ	534
POOL 27	POOL 69	
A3A1 219 QAIH NVVH AII HQQQ RQQQ	205 G13A 361 CSTI RAPP ASIV AGIG GQYR	535
A3B1 219 QAIH NVVH AII HQQQ RQQQ	206 G14 361 CSTI RAPP ASIV ASIG GQ	536
A3B3 219 QAIH NVVH AII HQQQ RQQQ	207 G15A 361 CSTT KAPF ASIV ADIG GQ	537
A4A 219 QAIH NVVH AII HHHQ KQQQ	208 G15C 361 CSTI NAPP ASIV AGIS GQ	538
A1A1 227 AII HQQQ KQQQ QPSS QVSF	209 G16A 361 CSTI NVPY ANID AGIG GQ	539
A1A6 227 AII HQQQ KQQQ QPSS SFQS	210 G11 1 PQQF FPLQ PQQS FLWQ SQQP	540
A1B2 227 AII HQQQ KQQQ QPSS QVSF	211 G11 9 PQQS FLWQ SQQP FPLQ PQQP	541
A1B10 227 AII HQQQ KQQQ PSSQ VSFQ	212 G11 17 SQQP FLQQ PQQP SPQP QQVV	542
POOL 28	POOL 70	
A1B13 227 AII HQQQ KQQQ EQKQ QLQQ	213 G11 25 PQQP SPQP QQVV QIIS PATP	543
A2A1 227 AII HQQQ KQQQ QQQQ QPLS	214 G11 33 QQVV QIIS PATP TTIP SAGK	544
A2B3 227 AII HQQQ HHHQ KQQQ QQQQ	215 G11 41 PATP TTIP SAGK PTSA PPPQ	545
A2B4 227 AII HQQQ HHHQ EQKQ QLQQ	216 G11 49 SAGK PTSA PPPQ QQQQ HQQL	546
A3A1 227 AII HQQQ RQQQ PSSQ VSLQ	217 G11 57 PPPQ QQQQ HQQL AQQQ IPVV	547
A3B1 227 AII HQQQ RQQQ LQQQ QQQQ	218 G11 65 HQQL AQQQ IPVV QPSI LQLQ	548
A3B3 227 AII HQQQ RQQQ EQKQ LQLQ	219 G11 73 IPVV QPSI LQLQ NPCK VFLQ	549
A4A 227 AII HHHQ KQQQ QPSS QVSY	220 G11 81 LQLQ NPCK VFLQ QQCS PVAM	550
POOL 29	POOL 71	
A1A1 235 KQQQ QPSS QVSY QQPL QQYP	221 G11 89 VFLQ QQCS PVAM PQRL ARSQ	551
A1A6 235 KQQQ QPSS QFSE QQPL QQYP	222 G11 97 PVAM PQRL ARSQ MLQQ SSCH	552
A1B2 235 KQQQ QLSS QVSF QQPL QQYP	223 G11 105 ARSQ MLQQ SSCII VMQQ QCCQ	553
A1B10 235 KQQQ PSSQ VSFQ QPQQ QYPL	224 G11 113 SSCH VMQQ QCCQ QLPQ IPQQ	554
A1B13 235 KQQQ EQKQ QLQQ QQQQ QQL	225 G11 121 QCCQ QLPQ IPQQ SRYQ AIRA	555
A2B4 235 HHHQ EQKQ QLQQ QQQQ QQL	226 G11 127B PQQ QCSR YEAJ RAIY YSH	556
A3A1 235 RQQQ PSSQ VSLQ QPQQ QYPS	227 G11 129 IPQQ SRYQ AIRA IIYS IIIQ	557
A3B1 235 QQQQ LQQQ QQQQ LQQQ QQQQ	228 G11 137 AIRA IIYS IIIQ EQQQ VQGS	558
POOL 30	POOL 72	
A4A 235 QQQQ QPSS QVSY QQPL QQYP	229 G11 145 IIIQ EQQQ VQGS IQSQ QQQP	559
A1B13 243 QLQQ QQQQ QQQL QQQQ KQQQ	230 G11 153 VQGS IQSQ QQQP QQLG QCVS	560
A1B13 251 QLQQ QQQQ QKQQ QQQP SQVS	231 G11 161 QQQP QOLQ QCVS QPQQ QSQQ	561
A2A1 260 QQQQ QQQQ QPLS QVSF QQQP	232 G11 169 QCVS QQQP QSQQ QLQG QPQQ	562
A2B1 260 QQQQ QQQQ QPLS QVCF QQQQ	233 G11 171 QSQQ QLQG QPQQ QQLA QGTF	563
A2B3 260 HHHQ QQQQ QQQQ QPLS QVFS	234 G11 185 QPQQ QQLA QGTF LQPH QIAQ	564
A3B1 260 QQQQ QQQQ QPSS QVSF QQQP	235 POOL 73	
A2A1 289 QPLS QVSF QQQP QQYP SGQG	236 G11 193 QGTF LQPH QIAQ LEVM TSI	565
POOL 31	G11 201 QIAQ LEVM TSI LRL PTMC	566
A2B1 289 QPLS QVCF QQQQ QQYP SGQG	237 G11 209 TSI LRL PTMC SVNV PLYR	567
A3B1 289 QVSS QVSF QQQP QQYP SSQV	238 G11 217 PTMC SVNV PLYR TTTS VPGF	568
A1A1 293 QVSF QQPL QQYP LGQQ SFRP	239 G11 225 PLYR TTTS VPGF VGTG VGA	569
A1A6 293 QPSF QQPL QQYP LGQQ SFRP	240 G111 1A 1 TTTR TFPI PTIS SNNN HHFR	570
A1B2 293 QVSF QQQP QQYP LGQQ SFRP	241 G111 1A 9 PTIS SNNN HHFR SNSN HHFH	571
A2A1 293 QVSF QQQP QQYP SGQQ SFQP	242 G111 1A 17 HHFR SNSN HHFH SNNN QFYR	572
A2B3 293 QVSF QQQP QQYP SGQQ SFQP	243 POOL 74	
POOL 32	244 G111 1A 25 HHFH SNNN QFYR NNNS PGHN	573
A2B5 293 QVSF QQQP QQYP SGQQ FFQP	G111 1A 33 QFYR NNNS PGHN NPLN NNNS	574
A3A1 293 QVSL QQQP QQYP SGQQ FFQP	245 G111 1A 41 PGHN NPLN NNNS PNNN SPSN	575
A3B1 293 QVSF QQQP QQYP SSQV SFQP	246 G111 1A 49 NNNS PNNN SPSN HHNN SPNN	576
A3B2 293 QVSF QQQP QQYP SSQG SFQP	247 G111 1A 57 SPSN HHNN SPNN NFQY HTHP	577
A4A 293 QVSY QQQP EQYP SGQV SFQS	248 G111 1A 65 SPNN NFQY HTHP SNHK NLPH	578
A1A1 301 QQYP LQQQ SFRP SQQN PQAQ	249 G111 1A 73 HTHP SNHK NLPH TNNI QQQQ	579
A1B2 301 QQYP LQQQ SFRP SQQN PQAQ	250 G111 1A 81 NLPH TNNI QQQQ PPFS QQQQ	580
POOL 33	251 POOL 75	
A2A1 301 QQYP SGQQ SFQP SQQN PQAQ	252 G111 1A 89 QQQQ PPFS QQQQ PPFS QQQQ	581
POOL 33	G111 1A 97 QQQQ PPFS QQQQ PVLP QQQP	582

A2B3 301 QQYP SGQQ FFQP SQQN PQAQ	253 GI11 1A 105 QQQQ PVLP QQSP FSQQ QQLV	583
A2B5 301 QQYP SGQQ FFQP FQQN PQAQ	254 GI11 1A 113 QQSP FSQQ QQLV LPQQ QQQQ	584
A3A1 301 QQYP SGQQ FFQP SQQN PQAQ	255 GI11 1A 121 QQLV LPQQ QQQQ QLVC QQIP	585
A3B1 301 QQYP SSQV SFQP SQLN PQAQ	256 GI11 1A 129 QQQQ QLVC QQIP IVQP SVLQ	586
A3B2 301 QQYP SSQG SFQP SQQN PQAQ	257 GI11 1A 137 QQIP IVQP SVLQ QLNP CKVP	587
A4A 301 EQYP SGQV SFQS SQQN PQAQ	258 GI11 1A 145 SVLQ QLNP CKVF LQQQ CSPV	588
A1B1 309 SFRP SQQN PLAQ GSVQ PQQL	259 POOL 76	
A1A1 309 SFRP SQQN PQAQ GSVQ PQQL	260 GI11 1A 153 CKVF LQQQ CSPV AMPO RLAR	589
POOL 34	GI11 1A 161 CSPV AMPO RLAR SQMW QSSS	590
A1A3 309 SFRP SQQN PQTQ GSVQ PQQL	261 GI11 1A 169 RLAR SQMW QSSS CHVM QQQC	591
A1B2 309 SFRP SQQN SQAQ GSVQ PQQL	262 GI11 1A 177 QSSS CHVM QQQC CQQL QQIP	592
A1B3 309 SFRP SQQN PQDQ GSVQ PQQL	263 GI11 1A 185 QQQC CQQL QQIP EQSR YEAI	593
A1B4 309 SFRP SQQN PRAQ GSVQ PQQL	264 GI11 1A 193 QQIP EQSR YEAI RAII YSII	594
A2A1 309 SFQP SQQN PQAQ GSVQ PQQL	265 GI11 1A 201 YEAI RAII YSII LQEQQ QQQF	595
A2B3 309 FFQP SQQN PQAQ GSFP PQQL	266 GI11 1A 209 YSII LQEQQ QQQF VQPQ QQQP	596
A2B5 309 FFQP FQQN PQAQ GSFP PQQL	267 POOL 77	
A3A1 309 FFQP SQQN PQAQ GSVQ PQQL	268 GI11 1A 217 QQQF VQPQ QQQP QSSG QGVS	597
Pool 35	GI11 1A 225 QQQP QSSG QGVS QSQQ QSQQ	598
A3B1 309 SFQP SQLN PQAQ GSVQ PQQL	269 GI11 1A 233 QGVS QSQQ QSQQ QLQQ CSFQ	599
A3B2 309 SFQP SQLN PQAQ GSVQ PQQL	270 GI11 1A 241 QSSQ QLQQ CSFQ QPQQ QLQQ	600
A4A 309 SFQS SQQN PQAQ GSVQ PQQL	271 GI11 1A 249 CSFQ QPQQ QLQQ QPQQ QQQQ	601
A1A1 317 PQAQ GSVQ PQQL PQFE EIRN	272 GI11 1A 257 QLQQ QPQQ QQQQ QLVL GTFL	602
A1A3 317 PQTQ GSVQ PQQL PQFE EIRN	273 GI11 1A 263 QQQQ QLVL GTFL QPHQ IAHL	603
A1A6 317 PQAQ GSVQ PQQL PQFE EIRN	274 GI11 1A 271 GTFL QPHQ IAHL EAVT SIAL	604
A1B1 317 PLAQ GSVQ PQQL PQFE EIRN	275 POOL 78	
POOL 36	276 GI11 1A 279 IAHL EAVT SIAL RTLP TMCS	605
A1B3 317 PQDQ GSVQ PQQL PQFB EIRN	GI11 1A 287 SIAL RTLP TMCS VNVP LYSA	606
A1B4 317 PRAQ GSVQ PQQL PQFE EIRN	277 GI11 1A 295 TMCS VNVP LYSA TTSV PFGV	607
A2B3 317 PQAQ GSFP PQQL PQFE EIRN	278 GI11 1A 303 LYSA TTSV PFGV GTGV GAY	608
A2B5 317 PQAQ GSFP PQQL PQFE EIRN	279 GI11 1B 26 SCIS GLER PWQQ QPLP PQQS	609
A3B1 317 PQAQ GSVQ PQQL POF A EIRN	280 GI11 1B 34 PWQQ QPLP PQQS FSQQ PPFS	610
A4A 317 PQAQ GSVQ PQQL POF EIRN	281 GI11 1B 42 PQQS FSQQ PPFS FSQQ QPLP	611
Pool 37	282 GI11 1B 50 PPFS FSQQ QPLP PQQS FSQQ	612
A1A1 325 PQQL PQFE EIRN LALQ TLPA	283 GI11 1B 58 QPLP QQPS FSQQ QPPF SQQQ	613
A1A6 325 PQQL PQFE EIRN ALQT LPAM	284 GI11 1B 66 FSQQ QPPF SQQQ PILS QOPP	614
A1B12 325 PQQL PQFE EIRN LARK	285 GI11 1B 74 SQQQ PILS QQPP FSQQ QQPV	615
A2A1 325 PQQL PQFE EIRN LALB TLPA	286 O 1A 17 ATAA RELN PSNK ELOQ PQQS	616
A2B5 325 PQQL PQFE EIRN LALQ TLPA	287 O 1A 25 PSNK ELQS PQQS FSYQ QQPV	617
A3B1 325 PQQL PQFA EIRN LALQ TLPA	288 O 1A 33 PQQS FSYQ QQPV PQQP YPQQ	618
A4A 325 PQQL PQFO EIRN LALQ TLPA	289 O 1A 41 QQPV PQQP YPQQ PYPS QQPY	619
A1A1 333 EIRN LALQ TLPA MCNV YIPP	290 O 1A 49 YPQQ PYPS QQPY PSQQ PPFT	620
POOL 38	Pool 79	
A1A3 333 EIRN LALQ TLPS MCNV YIPP	291 O 1A 57 QQPY PSQQ PPFT PQQQ FPEQ	621
A2A1 333 EIRN LALE TLPA MCNV YIPP	292 O 1A 65 PPFT PQQQ FPEQ SQQP FTQP	622
A3A1 333 EIRN LALQ TLPR MCNV YIPP	293 O 1A 73 FPEQ SQQP FTQP QQPFT QPQ	623
A1A1 341 TLPA MCNV YIPP YCTI APFG	294 O 1A 81 FTQP QQPFT QPQF QPQF	624
A1A3 341 TLPS MCNV YIPP YCTI APFG	295 O 1A 89 PIQP QQPFT QPQF QQPQ QPPF	625
A1B1 341 TLPA MCNV YIPP YCTI VPFG	296 O 1A 97 PQQQ QQPQ QPPF QPQQ PFPW	626
A1B4 341 TLPA MCNV YIPP YCAM APFG	297 O 1A 105 QPPF QPQQ FPPW QPQQ PFPQ	627
A1B9 341 TLPA MCNV YIPP YCTI TPFG	298 O 1A 113 PFPW QPQQ FPPW TQQS FPLQ	628
Pool 39	Pool 80	
A2A1 341 TLPA MCNV YIPP YCTI APVG	299 O 1A 121 PFPQ TQQS PPLQ QPQP FPQQ	629
A2B2 341 TLPA MCNV YIPP YCST TIAP	300 O 1A 129 FPLQ PQQP FPQQ PQQP FPQP	630
A3A1 341 TLPR MCNV YIPP YCST TIAP	301 O 1A 137 FPQQ PQQP FPQQ QLFP PQQS	631
A3A2 341 TLPR MCNV YIPP YCST TTAP	302 O 1A 145 FPQP QLFP PQQS EQU PQQL	632
A3B1 341 TLPA MCNV YIPP HCST TIAP	303 O 1A 153 PQQS EQU PQQL QQPQ PLQP	633
A1A1 349 YIPP YCTI APFG IFGT NYR	304 O 1A 161 PQQL QQPQ PLQP QQPQ PQQP	634
A1B1 349 YIPP YCTI VFVG IFGT NYR	305 O 1A 169 PLQP QQPQ PQQP QQPQ PQQP	635
A1B4 349 YIPP YCAM APFG IFGT NYR	306 O 1A 177 PQQP QQPQ PQQP VQPQ	636
Pool 40	Pool 81	
A1B5 349 YIPP YCTM APFG IFGT NYR	307 O 1A 185 PQQP QQPQ VQPQ QSFP QQQQ	637
A1B9 349 YIPP YCTI TPFG IFGT N	308 O 1A 193 VQPQ QSFP QQQQ QSQQ PFAQ	638
A2A1 349 YIPP YCTI APVG IFGT NYR	309 O 1A 201 QQQQ QSQQ PFAQ PQQL FPEL	639
A2B2 349 YIPP YCST TIAP VGIF GTN	310 O 1A 209 PFAQ PQQL FPEL QQPQ PQQP	640
A3A2 349 YIPP YCST TTAP FGIF GTN	311 O 1A 217 FPEL QQPQ PQQP QQPQ PLQP	641
A3B1 349 YIPP HCST TIAP FGIF GTN	312 O 1A 225 PQQP QQPQ PLQP QQPQ PQQP	642
A3B3 349 YIPP HCST TIAP FGIS GTN	313 O 1A 233 PLQP QQPQ PQQP QQPQ PQQP	643
A4D 350 IPYF CSTT IAPF GIFG TNYR	314 O 1A 241 PQQP QQPQ PQQP QQPQ PQQP	644
Pool 41	Pool 82	
A1B5 349 YIPP YCTM APFG IFGT NYR	315 O 1A 249 PQQP QQPQ PQQP QQPY PQQQ	645
A1B9 349 YIPP YCTI TPFG IFGT N	316 O 1A 257 PQQP QQPY PQQQ PYGS SLIS	646
A2A1 349 YIPP YCTI APVG IFGT NYR		
A2B2 349 YIPP YCST TIAP VGIF GTN		
A3A2 349 YIPP YCST TTAP FGIF GTN		
A3B1 349 YIPP HCST TIAP FGIF GTN		
A3B3 349 YIPP HCST TIAP FGIS GTN		
A4D 350 IPYF CSTT IAPF GIFG TNYR		
Pool 42	Pool 83	
G11A 17 GTAN MQVD PSSQ VQWP QQQP		
G12A 17 GTAN IQVD PSGQ VQWL QQQL		

G1A 17 ATAN MQVD PSGQ VPWP QQQP	317	O 1A	265 PQQQ PYGS SLTS IGGQ	647
G1B 19 MN IQVD PSGQ VPWP QQQP FP	318	O 1B	1 ARQL NPSD QELQ SPQQ LYPQ	648
GI4 17 ATAN MQAD PSGQ VQWP QQQP	319	O 1B	9 QELQ SPQQ LYPQ QPYP QQPY	649
GI5A 17 TTAN IQVD PSGQ VQWP QQQQ	320	O 1C	1 SRLL SPRG KELH TPQE QFPQ	650
GI5C 17 ATAN MQVD PSGQ VQWP QQQP	321	O 1C	9 KELH TPQE QFPQ QQQF PQPQ	651
GI7 20 QIVF PSGQ VQWP QQQQ PFP	322	O 1C	17 QFPQ QQQF PQPQ QFPQ	652
Pool 42				
GI1A 25 PSSQ VQWP QQQP VPQP HQPF	323			
GI2A 25 PSGQ VQWL QQQL VPQL QQPL	324			
GI3A 25 PSGQ VPWP QQQP FPQP HQPF	325			
GI4 25 PSCQ VQWP QQQP FLQP HQPF	326			
GI5A 25 PSGQ VQWP QQQQ PFPQ PQQP	327			
GI5C 25 PSGQ VQWP QQQP FRQP QQPF	328			
GI6A 25 PSGQ VQWP QQQP FPQP QQPF	329			
GI1A-33 QQQP VPQP HQPF SQQP QQTF	330			

*Position of N-terminal residue in α -, $\gamma 1$ -, $\gamma 2$ -, $\gamma 3$ -, or ω consensus sequence

Table 24. 652 synthetic peptides and ELISpot analysis with patients and gluten challenge

COELIAC SUBJECT	C14	C15	C16	C17	C18	C19	C20	C21	C22	C23	C24	C25	C26	C27	C28	
HLA-DQ	22	22	22	22	22	22	2X	2X	2X	2X	28	28	2X	2X	2X	
ANTIGEN CHALLENGE	WHEAT												RYE			
DURATION OF CHALLENGE (DAYS)	3	3	6	3	6	3	3	3	3	0.5	3	1	3	3	3	
DOMINANT PEPTIDE ELISPOT SFC	203	46	96	195	114	136	29	57	129	259	50	18	163	52	229	
BLANK ELISPOT SFC	1	2	4	0.5	3	1	1	4	3	2	1	2	1	2	6	

Peptide	Sequence
1	AVRFPVPLQQPQNPSSQQLPQ
2	MVRVPVPLQQPQNPSSQQPQ
3	MVRVPVPLQQPQNPSSQHQFQ
4	MVRVPVMPQLQPODPSSQQPQ
5	MVRVTVPLQQPQNPSSQQPQ
6	AVRVSVPQLQQPQNPSSQQPQ
7	AVRVPVPLQQPQNPSSQQPQ
8	AVRWFVPQLQQPQNPSSQQPQ
9	AVRVEFVPQLQQPQNPSSQQPQ
10	MVRVPVPLQQLQNPSSQQPQ
11	AVRVEFVPQPQNPSSQGPQ
12	AVRVEFVPQLQNPSSQQPQ
13	LQPQNPSSQQLPQEAVPLVQQ
14	LQPQNPSSQQQPQEAVPLVQQ
15	LQPQNPSSQQHPOEQAVPLVQQ
16	LQPODPSQQQPQEAVPLVQQ
17	LQPQNPSSQQQPQEAVPLVQQ
18	LQLQNPSSQQQPQEAVPLVQE
19	LQLQNPSSQQQPQEAVPLVQE
20	POPNPSPQPOPOGQAVPLVQQ
21	POPNPSPQPOPOQAVPLVQQ
22	LQPKNPSPQQQPQEAVPLVQQ
23	LQPQNPSSQQQPQEAVPLMQQ
24	QLPQEAVFLVQQQQFLGQQQ
25	QHPQEAVFLVQQQQFLGQQQ
26	QQPQEAVFLVQQQQFLGQQQ
27	QQPQEAVFLVQQQQFLGQQQ
28	QQPQEAVFLVQQQQFFGQQQ
29	QQPKQAVFLVQQQQFFGQQQ
30	QQPQEAVFLVQQQQFFGQQQ
31	PQPQGQAVFLVQQQQFFGQQQ
32	PQPQRQAVFLVQQQQFFGQQQ
33	QQPQEAVFLMQQQQQFFGQQQ
34	LVQQQQFLGQQQPFPPQQPY
35	LVQQQQFLGQQQSFPPQQPY
36	LVQQQQFLGQQQPFPPQQPY
37	LVQQQQFFGQQQPFPPQQPY
38	LVQEQQFFGQQQPFPPQQPY
39	LVQQQQFFGQQQPFPPQQPY
40	LMDQQQQPFPGQQQSFPPQQP
41	LMDQQQQPFPGQQQERFPQQP
42	GOQQPFPPDQPKPQDPEPS
43	GOQQPFPPDQPKPQDPEPSQ

99 LPYPOPQPFPRPQQPYPOSOP
100 LPYPOPQPFPRPQQSYQPQOP
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70.1 to 100

40.1 to 70

25.1 to 40

10.1 to 25

5.1 to 10

65

33 x 81

CLAIMS

1. A method of preventing or treating coeliac disease comprising administering to an individual at least one agent selected from:
 - 5 (a) a peptide comprising at least one epitope comprising a sequence selected from the group consisting of SEQ ID NOs:18-22, 31-36, 39-44, and 46, and equivalents thereof; and
 - (b) an analogue of (a) which is capable of being recognised by a T cell receptor that recognises the peptide of (a) and which is not more than 50 amino acids
 - 10 in length; and
 - (c) optionally, in addition to the agent selected from (a) and (b), a peptide comprising at least one epitope comprising a sequence selected from SEQ ID NO:1 and SEQ ID NO:2.
- 15 2. A method of claim 1 wherein the agent is HLA-DQ2-restricted.
3. A method of claim 1 wherein the agent is HLA-DQ8-restricted.
4. A method of claim 1 wherein one agent is HLA-DQ2-restricted and a second
- 20 agent is HLA-DQ8-restricted.
5. A method of claim 1 wherein the agent comprises a wheat epitope.
6. A method of claim 1 wherein one agent comprises a wheat epitope and one
- 25 agent comprises a rye epitope.
7. A method of claim 1 wherein one agent comprises a wheat epitope and one agent comprises a barley epitope.
- 30 8. A method of claim 1 wherein one agent comprises a rye epitope and one agent comprises a barley epitope.

9. A method of claim 1 wherein one agent comprises a wheat epitope, one agent comprises a barley epitope, and one agent comprises a rye epitope.

10. A method of claim 1 wherein a single agent comprises a wheat epitope, a barley epitope, and a rye epitope.

11. A method of preventing or treating coeliac disease comprising administering to an individual a pharmaceutical composition comprising an agent as defined in claim 1 and a pharmaceutically acceptable carrier or diluent.

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12. A method of preventing or treating coeliac disease comprising administering to an individual a pharmaceutical composition comprising an antagonist of a T cell which has a T cell receptor as defined in claim 1, and a pharmaceutically acceptable carrier or diluent.

15

13. A method of preventing or treating coeliac disease comprising administering to an individual a composition for tolerising an individual to a gliadin protein to suppress the production of a T cell or antibody response to an agent as defined in claim 1, which composition comprises an agent as defined in claim 1.

20

14. A method of preventing or treating coeliac disease comprising: diagnosing coeliac disease in an individual by either:

a) contacting a sample from the host with at least one agent selected from:

25

i) a peptide comprising at least one epitope comprising a sequence selected from the group consisting of: SEQ ID NOS:18-22, 31-36, 39-44, and 46, and equivalents thereof; and

30

ii) an analogue of i) which is capable of being recognised by a T cell receptor that recognises i) and which is not more than 50 amino acids in length; and

iii) optionally, in addition to the agent selected from i) and ii),
a peptide comprising at least one epitope comprising a
sequence selected from SEQ ID NOS:1 and 2; and

determining *in vitro* whether T cells in the sample recognise the agent;
5 recognition by the T cells indicating that the individual has, or is
susceptible to, coeliac disease; or

b) administering an agent as defined in claim 1 and determining *in*
10 *vivo* whether T cells in the individual recognise the agent, recognition of the
agent indicating that the individual has or is susceptible to coeliac disease;

and

administering to an individual diagnosed as having, or being susceptible to,
coeliac disease a therapeutic agent for preventing or treating coeliac disease.

15. Use of an agent for the preparation of a medicament for treating or preventing
15 coeliac disease, wherein the agent comprises:

(a) a peptide comprising at least one epitope comprising a sequence selected
from the group consisting of SEQ ID NOs:18-22, 31-36, 39-44, and 46, and
equivalents thereof; and

20 (b) an analogue of (a) which is capable of being recognised by a T cell
receptor that recognises the peptide of (a) and which is not more than 50 amino acids
in length; and

(c) optionally, in addition to the agent selected from (a) and (b), a peptide
comprising at least one epitope comprising a sequence selected from SEQ ID NO:1
and SEQ ID NO:2.

25

16. A use of claim 15 wherein the agent is HLA-DQ2-restricted.

17. A use of claim 15 wherein the agent is HLA-DQ8-restricted.

30 18. A use of claim 15 wherein one agent is HLA-DQ2-restricted and a second
agent is HLA-DQ8-restricted.

19. A use of claim 15 wherein the agent comprises a wheat epitope.
20. A use of claim 15 wherein one agent comprises a wheat epitope and one agent comprises a rye epitope.
5
21. A use of claim 15 wherein one agent comprises a wheat epitope and one agent comprises a barley epitope.
22. A use of claim 15 wherein one agent comprises a rye epitope and one agent comprises a barley epitope.
10
23. A use of claim 15 wherein one agent comprises a wheat epitope, one agent comprises a barley epitope, and one agent comprises a rye epitope.
- 15 24. A use of claim 15 wherein a single agent comprises a wheat epitope, a barley epitope, and a rye epitope.
25. A use of claim 15 wherein the agent is present within a pharmaceutical composition comprising a pharmaceutically acceptable carrier or diluent.
20
26. A use of claim 15 wherein the agent is present within a pharmaceutical composition comprising an antagonist of a T cell which has a T cell receptor as defined in claim 15, and a pharmaceutically acceptable carrier or diluent.
- 25 27. A use of claim 15 wherein the agent is present within a composition for tolerising an individual to a gliadin protein to suppress the production of a T cell or antibody response to an agent as defined in claim 1.
28. An agent as defined in claim 1, optionally in association with a carrier, for
30 use in a method of treating or preventing coeliac disease by tolerising T cells which recognise the agent.

29. An antagonist of a T cell which has a T cell receptor as defined in claim 1, optionally in association with a carrier, for use in a method of treating or preventing coeliac disease by antagonising such T cells.

5 30. An agent as defined in claim 1 or an analogue that binds an antibody that binds to an epitope of an agent as defined in claim 1 for use in a method of treating or preventing coeliac disease in an individual by tolerising the individual to prevent the production of such an antibody.

10 31. A protein that comprises a sequence which is able to bind to a T cell receptor, which T cell receptor recognises an agent as defined in claim 1, and which sequence is able to cause antagonism of a T cell that carries such a T cell receptor.

32. An agent as defined in claim 1 or an antagonist as defined in claim 12.

15 33. A pharmaceutical composition comprising an agent as defined in claim 1 or an antagonist as defined in claim 12 and a pharmaceutically acceptable carrier or diluent.

20 34. A composition for tolerising an individual to a gliadin protein to suppress the production of a T cell or antibody response to an agent as defined in claim 1, which composition comprises an agent as defined in claim 1.

25 35. A composition for antagonising a T cell response to an agent as defined in claim 1, which composition comprises an antagonist as defined in claim 12.

36. A mutant gliadin protein whose wild-type sequence can be modified by a transglutaminase to a sequence which is an agent as defined in claim 1, which mutant gliadin protein comprises a mutation which prevents its modification by a transglutaminase to a sequence which is an agent as defined in claim 1; or a fragment of such a mutant gliadin protein which is at least 15 amino acids long and which comprises the mutation.

37. A polynucleotide that comprises a coding sequence that encodes a protein or fragment as defined in claim 36 or 31.

38. A polynucleotide according to claim 37 that additionally comprises one or 5 more regulatory sequences operably linked to the coding sequence, which regulatory sequences are capable of securing the expression of the coding sequence in a cell.

39. A polynucleotide according to claim 38 wherein the regulatory sequence(s) allow expression of the coding sequence in a prokaryotic or mammalian cell.

10 40. A polynucleotide according to any one of claims 37 to 39 which is a vector or which is in the form of a vector.

41. A cell comprising a polynucleotide as defined in any one of claims 37 to 40 15 or which has been transformed with such a polynucleotide.

42. A cell according to claim 41 which is a prokaryotic cell or a mammalian cell.

43. A mammal that expresses a T cell receptor as defined in claim 1.

20 44. A method of diagnosing coeliac disease, or susceptibility to coeliac disease, in an individual comprising:
(a) contacting a sample from the host with at least one agent selected from
(i) a peptide comprising at least one epitope comprising a sequence selected from the group consisting of: SEQ ID NOS:18-22, 31-36, 39- 25 44, and 46, and equivalents thereof; and
(ii) an analogue of (i) which is capable of being recognised by a T cell receptor that recognises (i) and which is not more than 50 amino acids in length; and
(iii) optionally, in addition to the agent selected from (i) and (ii), a peptide comprising at least one epitope comprising a sequence selected from SEQ ID NOS:1 and 2; and

30

(b) determining *in vitro* whether T cells in the sample recognise the agent; recognition by the T cells indicating that the individual has, or is susceptible to, coeliac disease.

5 45. Use of an agent as defined in claim 44 for the preparation of a diagnostic means for use in a method of diagnosing coeliac disease, or susceptibility to coeliac disease, in an individual, said method comprising determining whether T cells of the individual recognise the agent, recognition by the T cells indicating that the individual has, or is susceptible to, coeliac disease.

10

46. A method or use according to claim 44 or 45 wherein the agent is an analogue (iii) which comprises (i) or (ii) bound to (a) an HLA molecule, or (b) a fragment of an HLA molecule capable of binding (i) or (ii).

15 47. A method or use according to claim 46 wherein the HLA molecule or fragment is in a complex comprising four HLA molecules or fragments of HLA molecules.

20 48. Use according to claim 45, 46 or 47 wherein the method comprises administering the agent to the skin of an individual and detecting the presence of inflammation at the site of administration, the detection of inflammation indicating that the T cells of the individual recognise the agent.

25 49. A method according to claim 44, 46 or 47 wherein the sample is blood sample.

50. A method according to claim 44, 46, 47 or 49 wherein the T cells are not restimulated in antigen specific manner *in vitro* before the said determining.

30 51. A method or use according to any one claims 44-50 in which the recognition of the agent by the T cells is determined by detecting the secretion of a cytokine from the T cells.

52. A method or use according to claim 51 in which the cytokine is IFN- γ .

53. A method or use according to claim 51 or claim 52 in which the cytokine is detected by allowing the cytokine to bind to an immobilised antibody specific to the cytokine and then detecting the presence of the antibody/cytokine complex.

54. A method or use according to any one of claims 44 to 50 wherein said determining is done by measuring whether the agent binds the T cell receptor.

10 55. A method for identifying an analogue as defined in a claim 44, 46 or 47 comprising determining whether a candidate substance is recognised by a T cell receptor that recognises an epitope comprising sequence as defined in claim 44, recognition of the substance indicating that the substance is an analogue.

15 56. A method of diagnosing coeliac disease, or susceptibility to coeliac disease, in an individual comprising determining the presence of an antibody that binds to an epitope of an epitope comprising sequence as defined in claim 44 in a sample from the individual, the presence of the antibody indicating that the individual has, or is susceptible to, coeliac disease.

20 57. A method of determining whether a composition is capable of causing coeliac disease comprising determining whether a protein capable of being modified by a transglutaminase to an oligopeptide sequence as defined in claim 44 is present in the composition, the presence of the protein indicating that the composition is capable of causing coeliac disease.

25 58. A method according to claim 57 wherein the said determining is done by contacting the composition with an antibody specific for the sequence which is capable of being modified to the oligopeptide sequence, binding of the antibody to a protein in the composition indicating the composition is capable of causing coeliac disease.

30

59. A method of identifying an antagonist of a T cell, which T cell recognises an agent as defined in claim 1, comprising contacting a candidate substance with the T cell and detecting whether the substance causes a decrease in the ability of the T cell to undergo an antigen specific response, the detecting of any such decrease in said ability indicating that the substance is an antagonist.

60. A kit for carrying out a method or use according to any one of claims 44 to 54 comprising an agent as defined in claim 44, 46 or 47 and a means to detect the recognition of the peptide by the T cell.

10

61. A kit according to claim 60 wherein the means to detect recognition comprises an antibody to IFN- γ .

15

62. A kit according to claim 61 wherein the antibody is immobilised on a solid support and optionally the kit also comprises a means to detect the antibody/IFN- γ complex.

20

63. Use of an agent or antagonist as defined in claim 62 or a wild type sequence as defined in claim 36 to produce an antibody specific to the agent, antagonist or wild type sequence.

64. Use of a mutation in an epitope of a gliadin protein, which epitope is as defined in claim 44, to decrease the ability of the gliadin protein to cause coeliac disease.

25

65. Method of identifying a product which is therapeutic for coeliac disease comprising administering a candidate substance to a mammal as defined in claim 43 which has, or which is susceptible to, coeliac disease and determining whether substance prevents or treats coeliac disease in the mammal, the prevention or treatment of coeliac disease indicating that the substance is a therapeutic product.

66. A therapeutic product as identified in the method of claim 65 for use in a method of preventing or treating coeliac disease.

67. A method of diagnosing coeliac disease, or susceptibility to coeliac disease in 5 an individual comprising administering an agent as defined in claim 44 and determining *in vivo* whether T cells in the individual recognise the agent, recognition of the agent indicating that the individual has or is susceptible to coeliac disease.

68. A cell according to claim 41 which is a cell of a graminaceous 10 monocotyledonous species.

69. A cell according to claim 68 which is a cell of wheat, maize, oats, rye, rice, barley, triticale, sorghum, or sugar cane.

15 70. A process for the production of a protein encoded by a coding sequence as defined in claim 37 which process comprises:

(a) cultivating a cell according to any one of claims 41, 42, 68 or 69 under conditions that allow the expression of the protein; and optionally
(b) recovering the expressed protein.

20

71. A method of obtaining a transgenic plant cell comprising:

(a) transforming a plant cell with a vector according to claim 40 to give a transgenic plant cell.

25 72. A method of obtaining a first-generation transgenic plant comprising:

(b) regenerating a transgenic plant cell transformed with a vector according to claim 40 to give a transgenic plant.

73. A method of obtaining a transgenic plant seed comprising:

30 (c) obtaining a transgenic seed from a transgenic plant obtainable by step
(b) of claim 72.

74. A method of obtaining a transgenic progeny plant comprising obtaining a second-generation transgenic progeny plant from a first-generation transgenic plant obtainable by a method according to claim 72, and optionally obtaining transgenic plants of one or more further generations from the second-generation progeny plant
5 thus obtained.

75. A method according to claim 74 comprising:

(d) obtaining a transgenic seed from a first-generation transgenic plant obtainable by the method according to claim 73, then obtaining a second-generation
10 transgenic progeny plant from the transgenic seed;
and/or
(e) propagating clonally a first-generation transgenic plant obtainable by the method according to claim 72 to give a second-generation progeny plant;
and/or
15 (f) crossing a first-generation transgenic plant obtainable by a method according to claim 72 with another plant to give a second-generation progeny plant;
and optionally
(g) obtaining transgenic progeny plants of one or more further generations from the second-generation progeny plant thus obtained.

20

76. A transgenic plant cell, plant, plant seed or progeny plant obtainable by a method according to any one of claims 71 to 75.

25 77. A transgenic plant or plant seed comprising plant cells according to claim 68 or 69.

78. A transgenic plant cell callus comprising plant cells according to claim 68 or 69 obtainable from a transgenic plant cell, first-generation plant, plant seed or progeny as defined in any one of claims 68, 69, or 71 to 75.

30

79. A plant or callus according to any one of claims 76 to 78 which is of a species as defined in claim 68 or 69.

80. A method of obtaining a crop product comprising harvesting a crop product from a plant according to any one of claims 76 to 79 and optionally further processing the harvested product.

5 81. A method according to claim 80 wherein the plant is a wheat plant and the harvested crop product is grain; optionally further processed into flour or another grain product.

82. A crop product obtainable by a method according to claim 80 or 81.

10

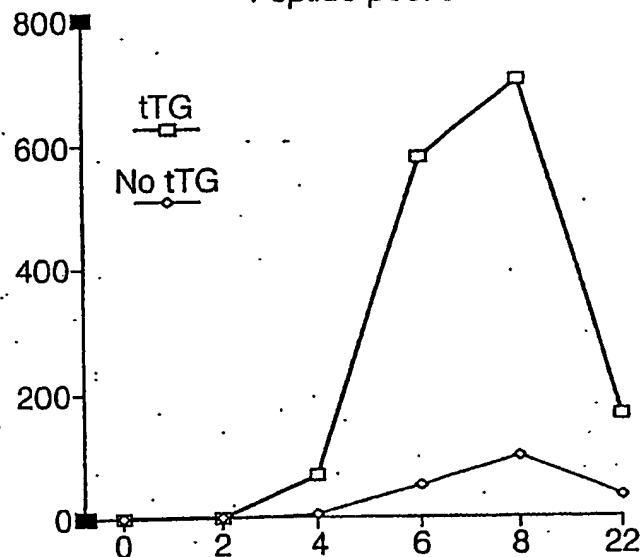
83. A food that comprises a protein as defined in any claim 31 or 36.

84. A food according to claim 83 in which a protein as defined in claim 31 or 36 is used instead of wild-type gliadin.

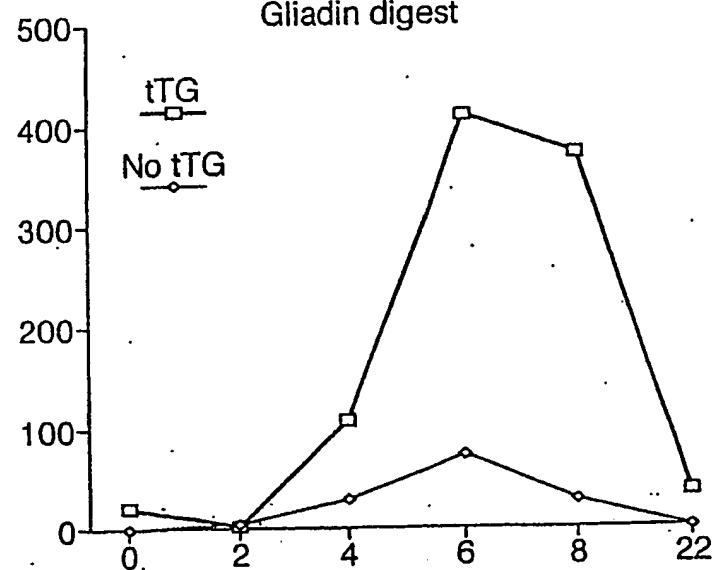
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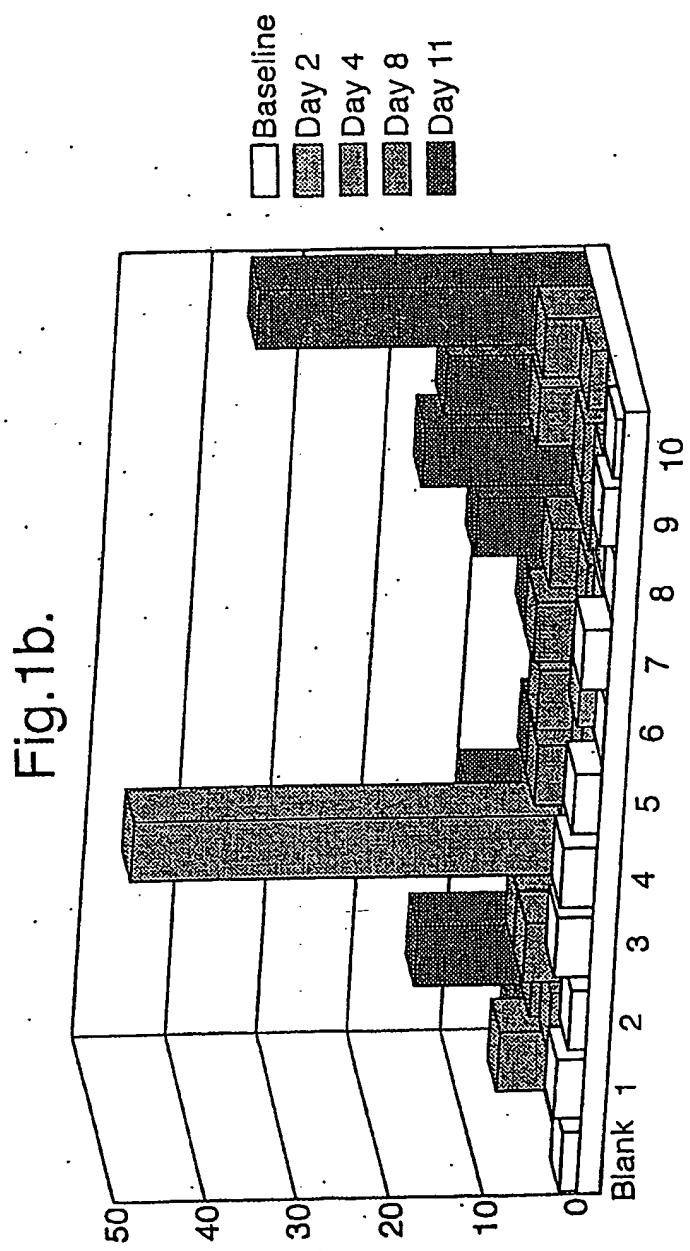
Fig. 1a.

Peptide pool 3



Gliadin digest





3/47

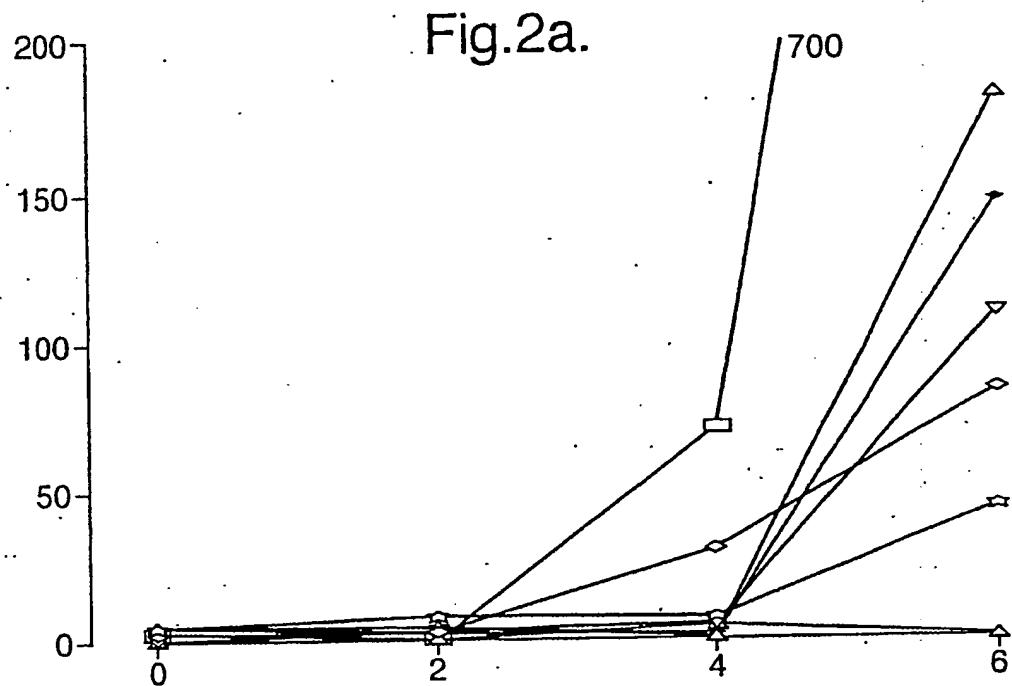
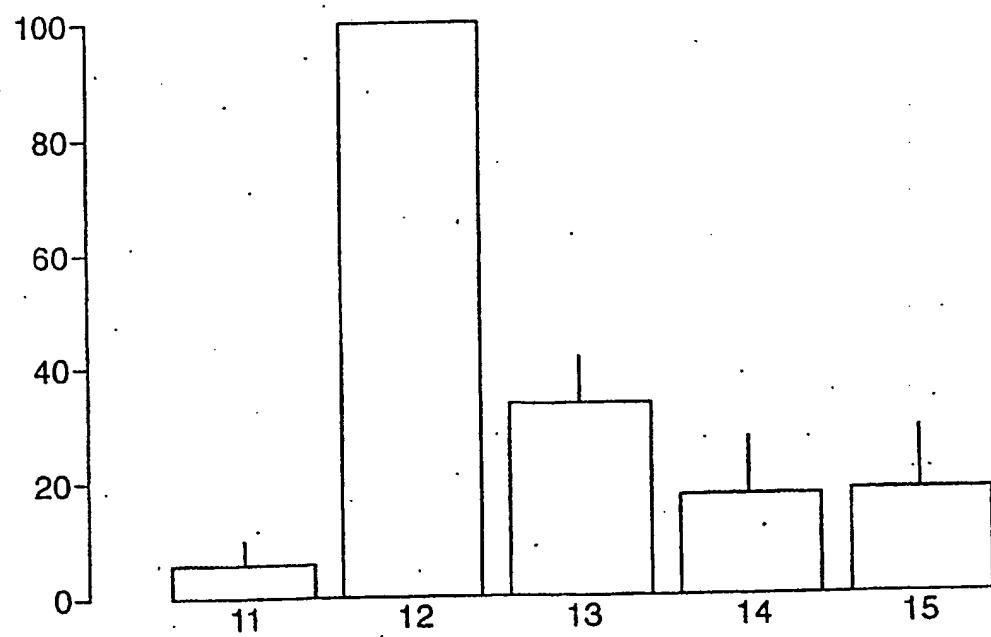


Fig.2b.



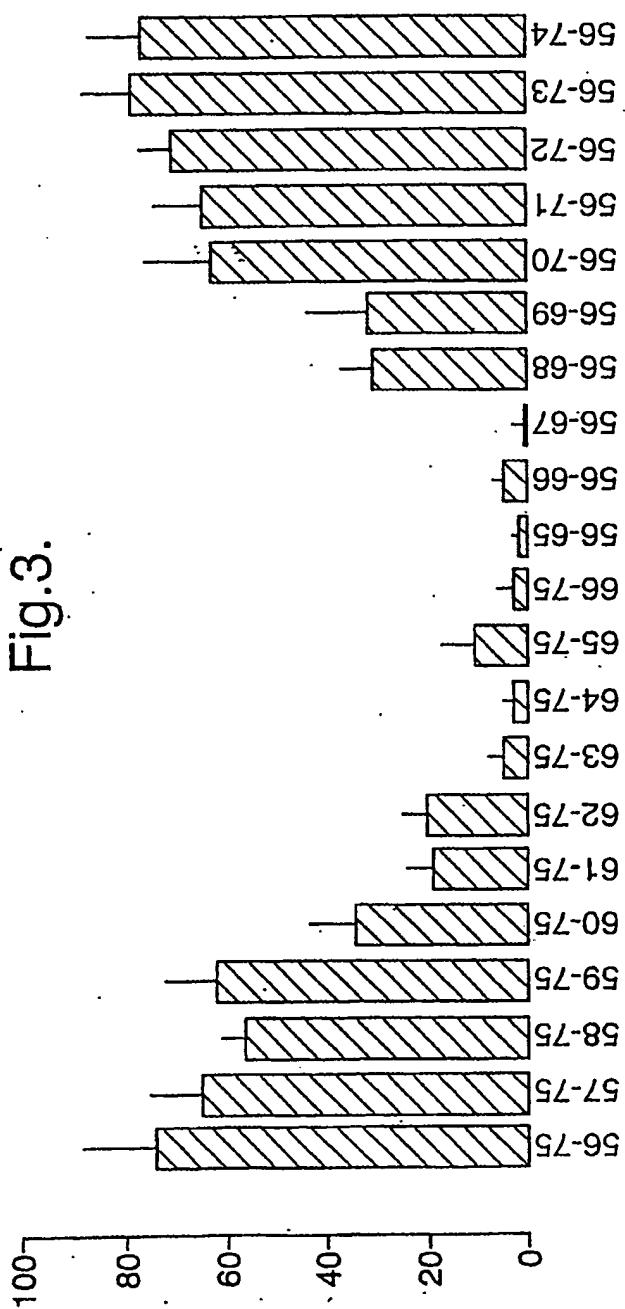


Fig.4a.

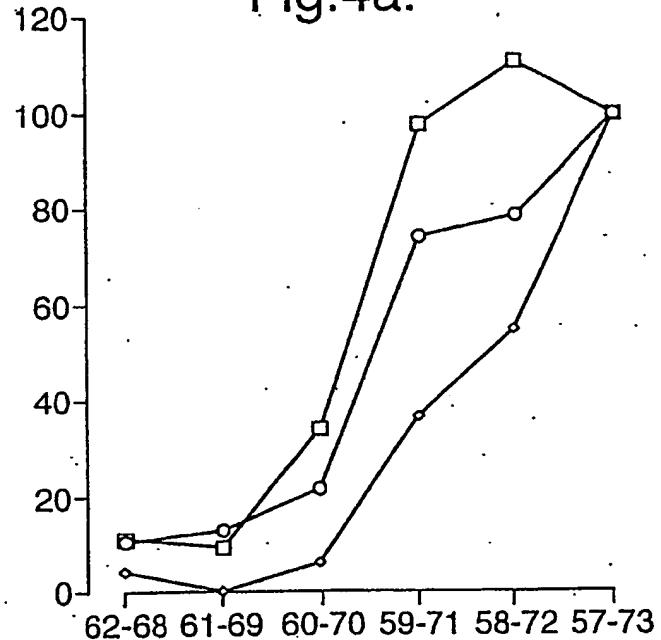
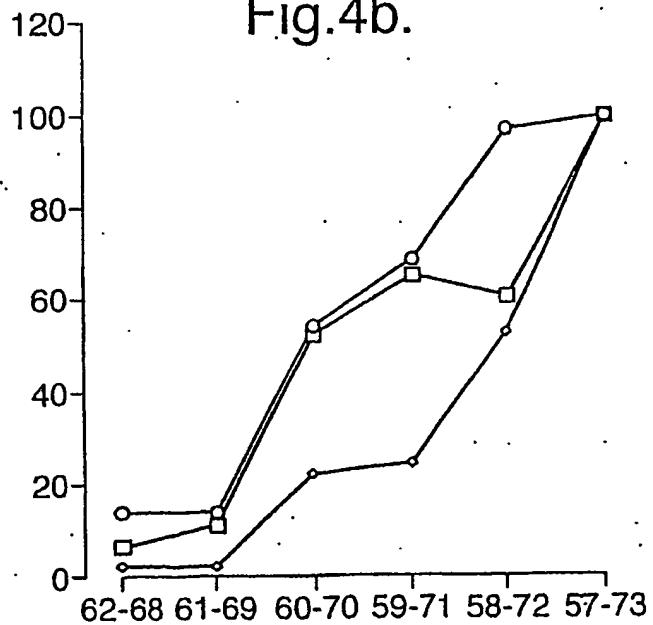


Fig.4b.



6/47

Fig.5.

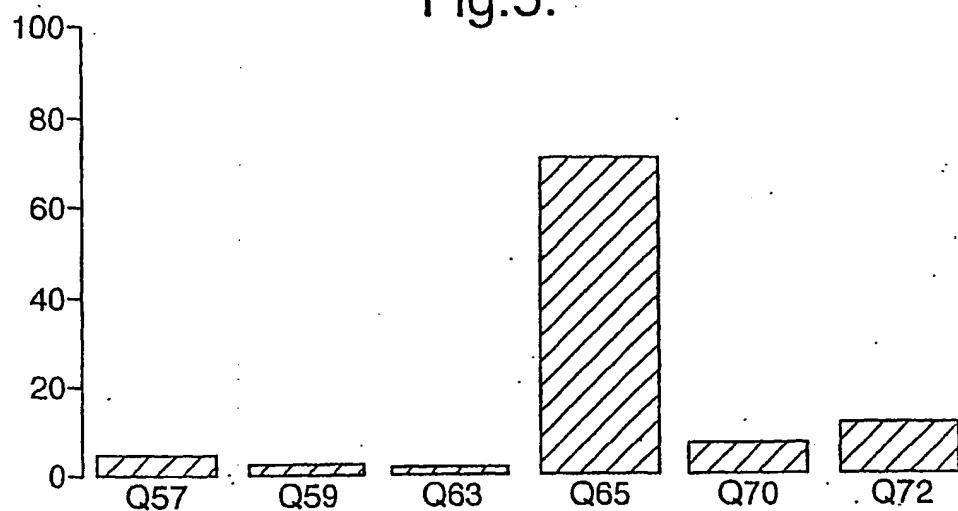
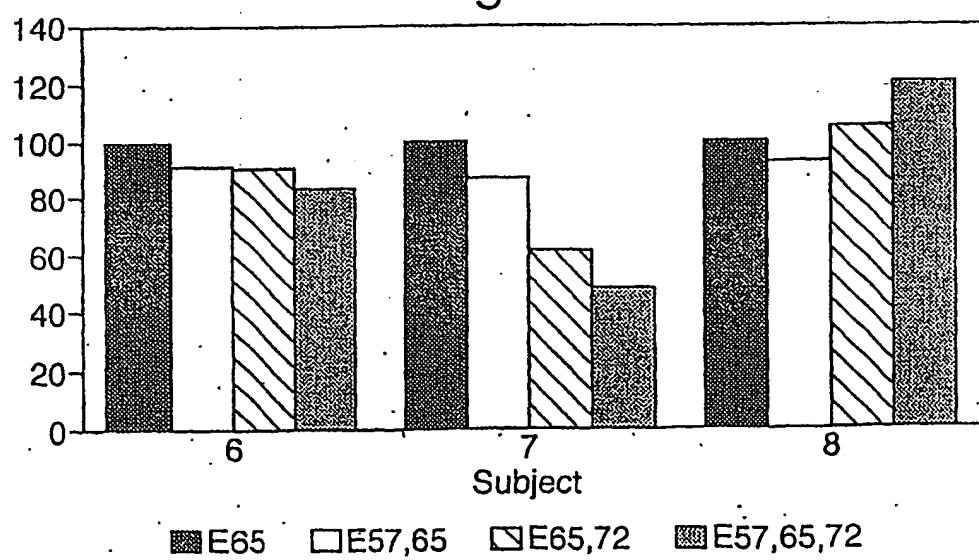


Fig.6.



7/47

Fig.7a.

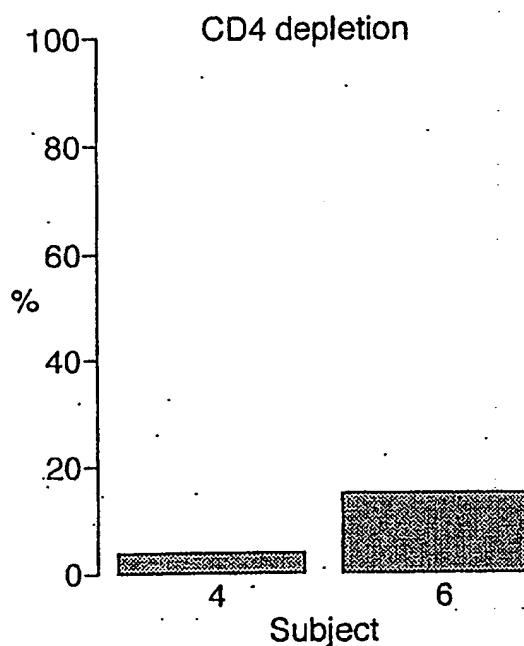
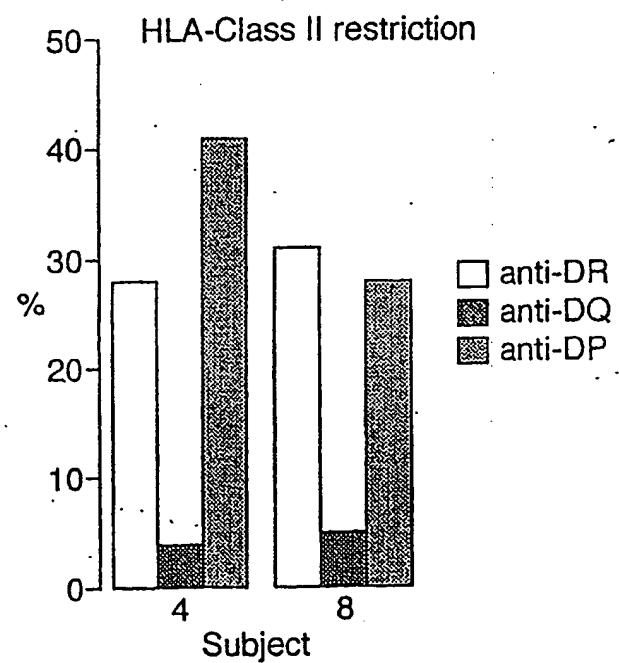


Fig.7b.



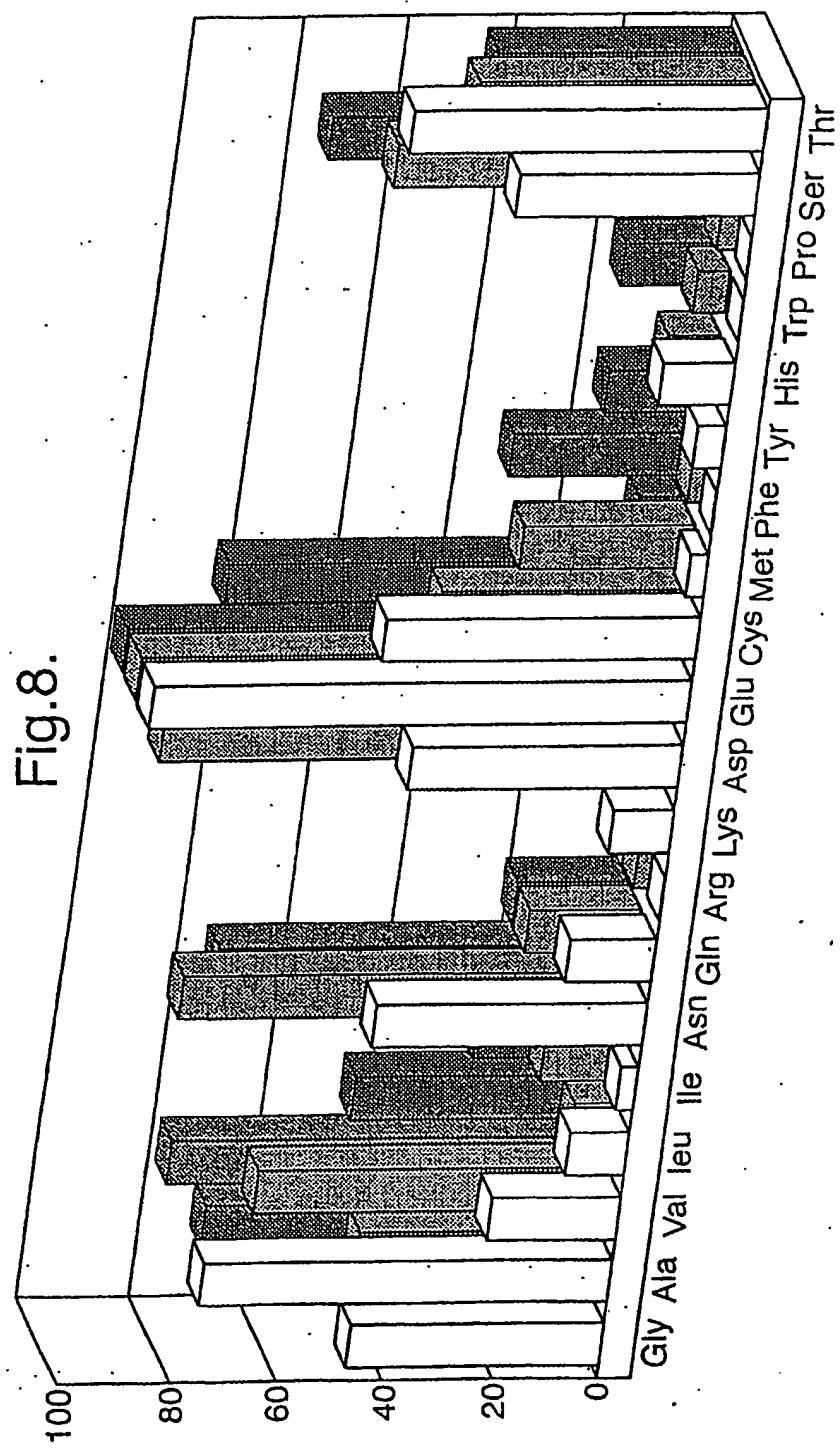


Fig.9.

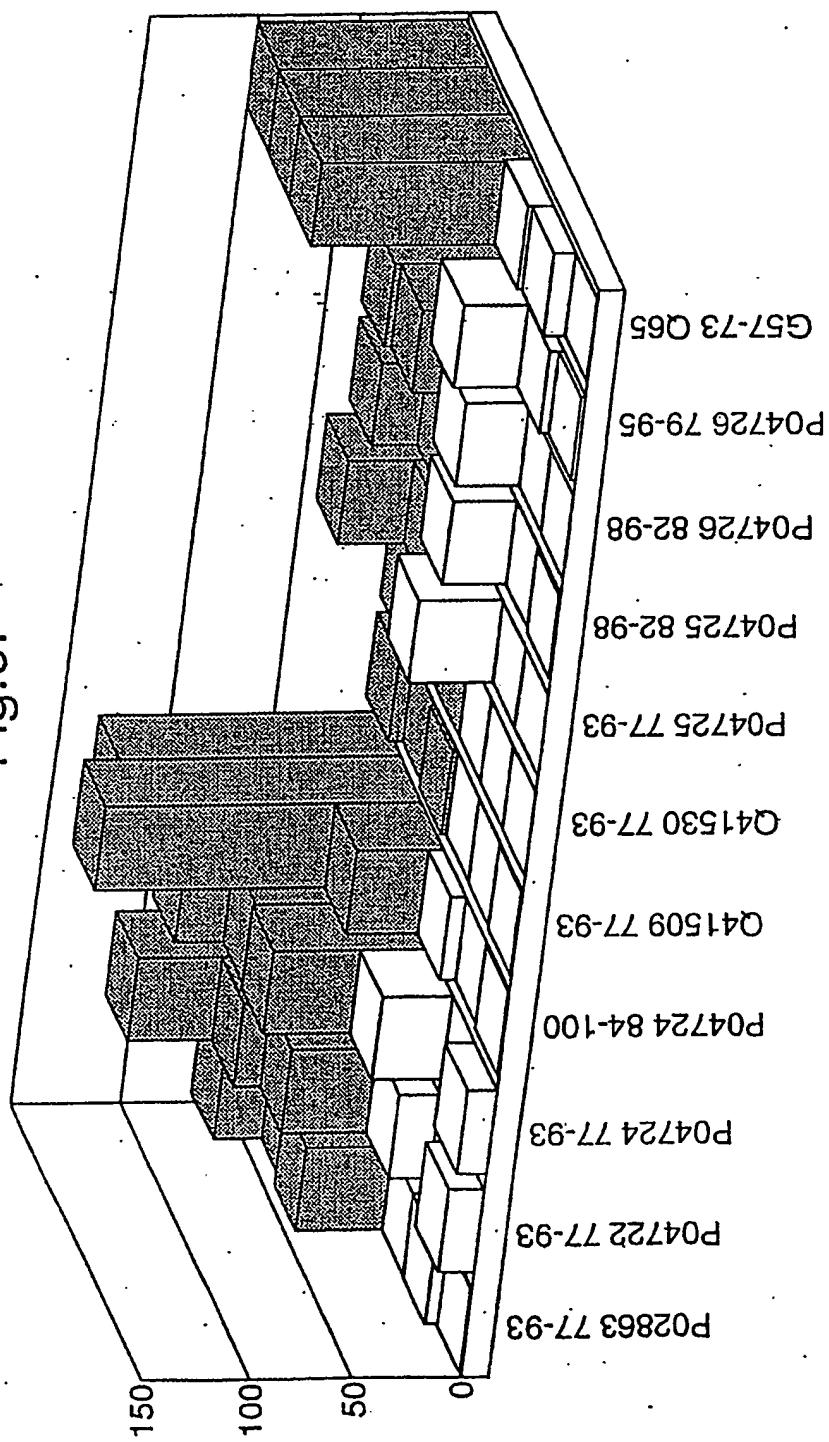


Fig.10.

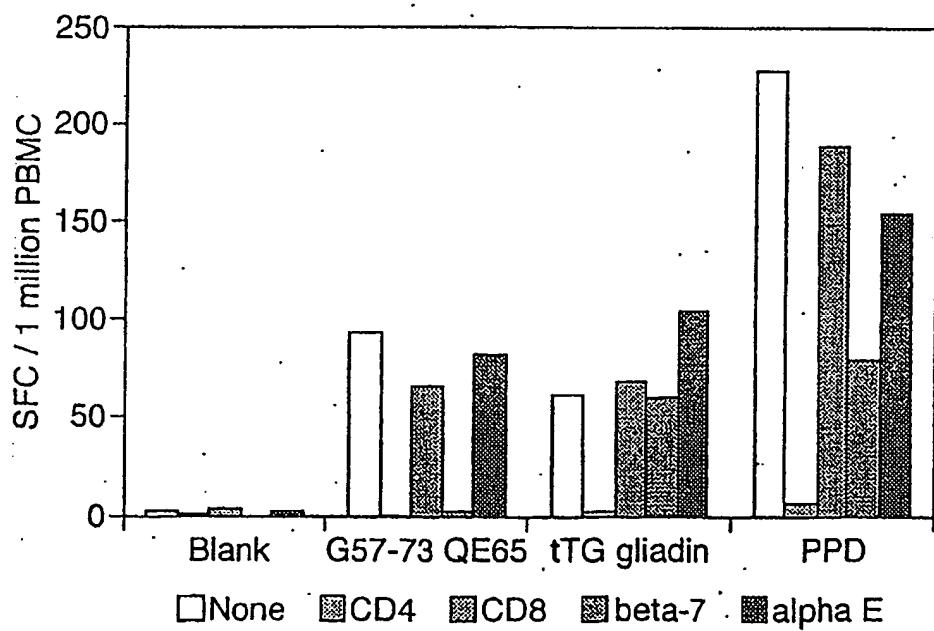
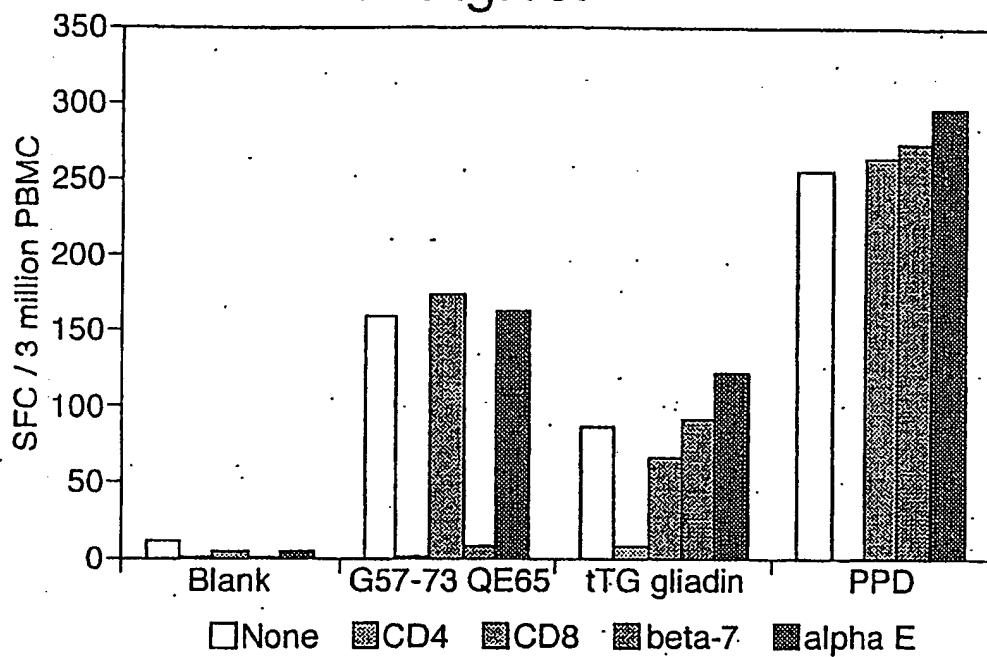


Fig.11.

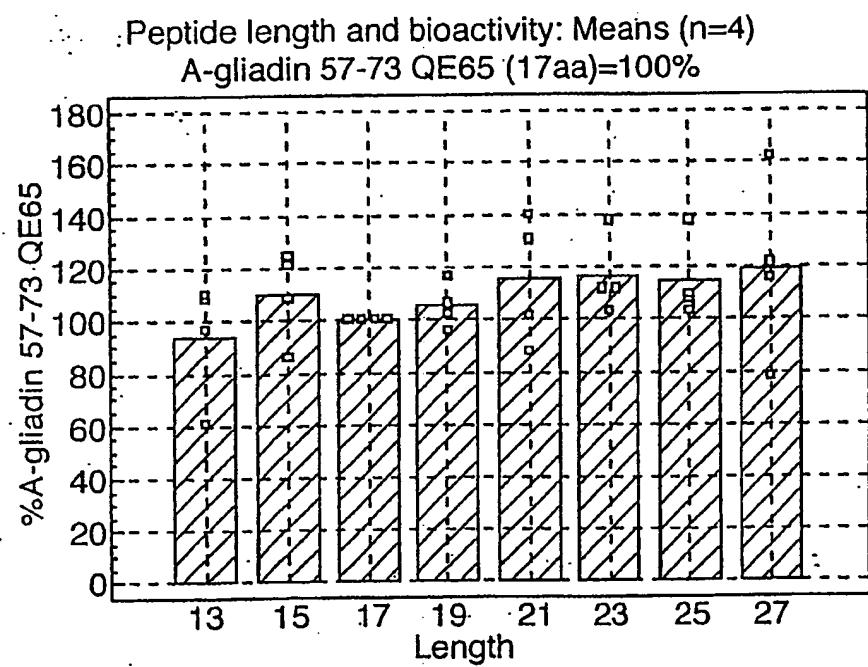


Fig.12a.

Dose response to A-gliadin 57-73 QE65:
 QLQPFPQPELPYPQPQPS.

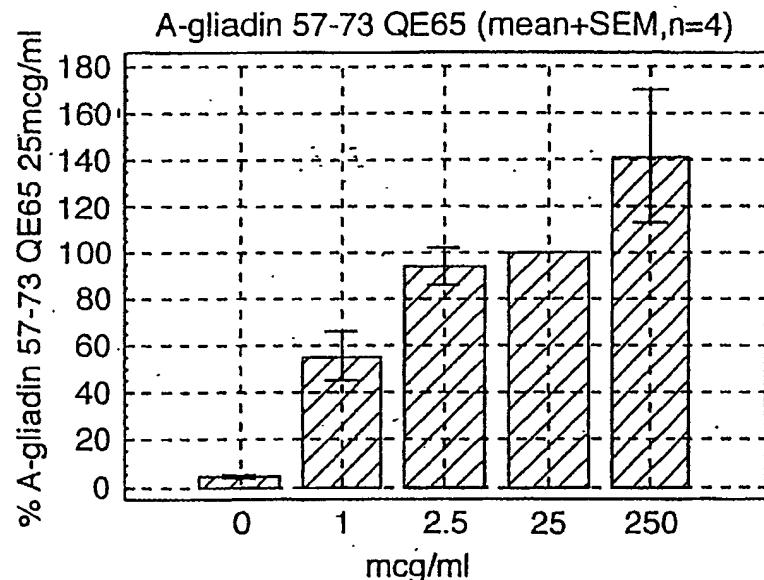


Fig.12b.

Dose response to GDA4_WHEAT P04724 84-100 QE92:
 PQLPYPQPELPYPQPQPS.

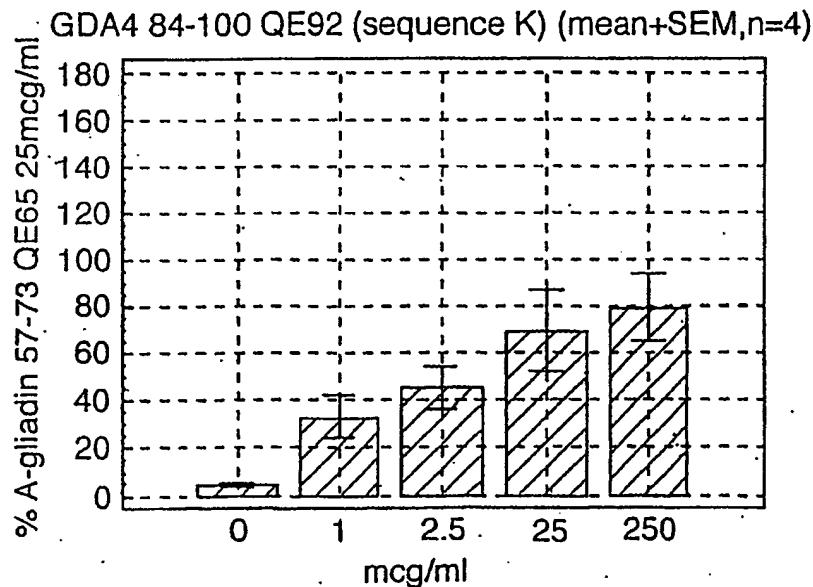


Fig.12c.

Dose response to A-gliadin 57-73:
 QLQPFPQPQLPYPQPQPS (2.5, 25 & 250 mcg/ml),
 and A-gliadin 57-73 (25 mcg/ml) + tTG treatment.

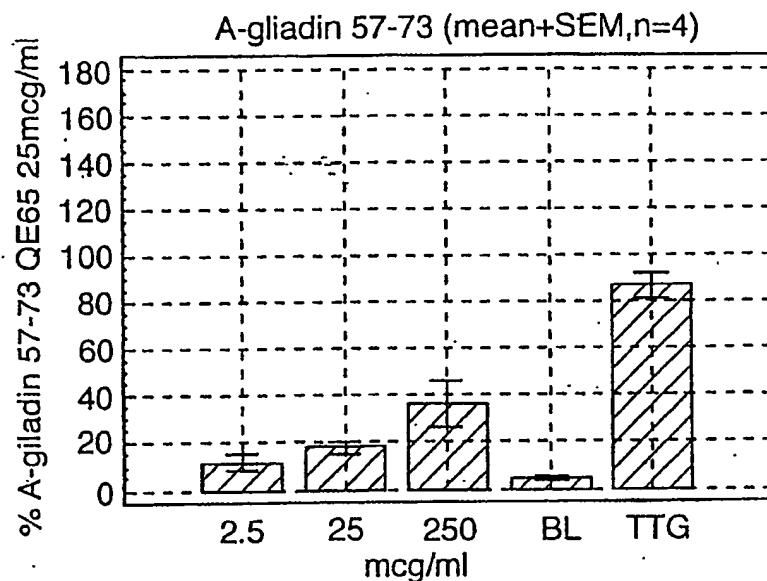


Fig.12d.

Dose response to GDA4_WHEAT P04724 84-100:
 PQLPYPQPQLPYPQPQPS (2.5, 25 & 250 mcg/ml),
 and P04724 84-100 (25 mcg/ml) + tTG treatment.

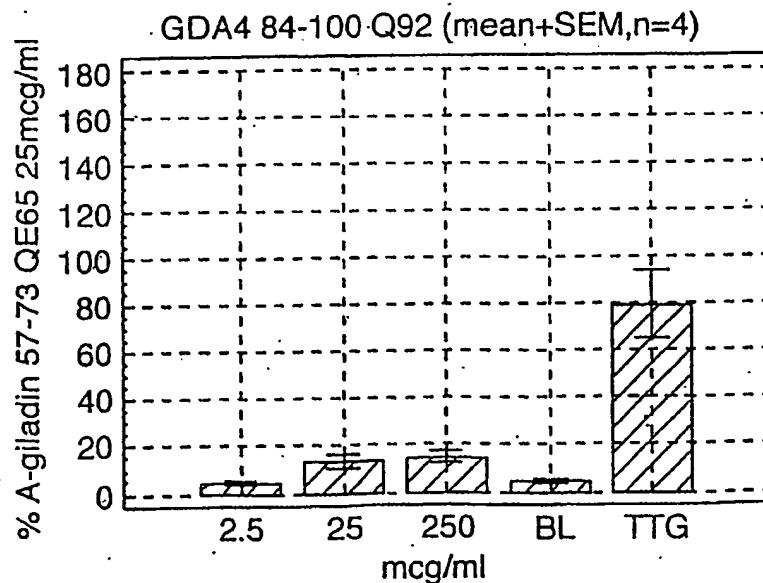


Fig.12e.

Dose response to the DQ2-restricted α gliadin T cell epitope A-gliadin 57-68 QE65:
QLQPFPQPELPY (E65) (2.5, 25 & 250 mcg/ml),
and A-gliadin 57-68: QLQPFPQPQLPY (Q65)
(25 mcg/ml) +/- tTG treatment.

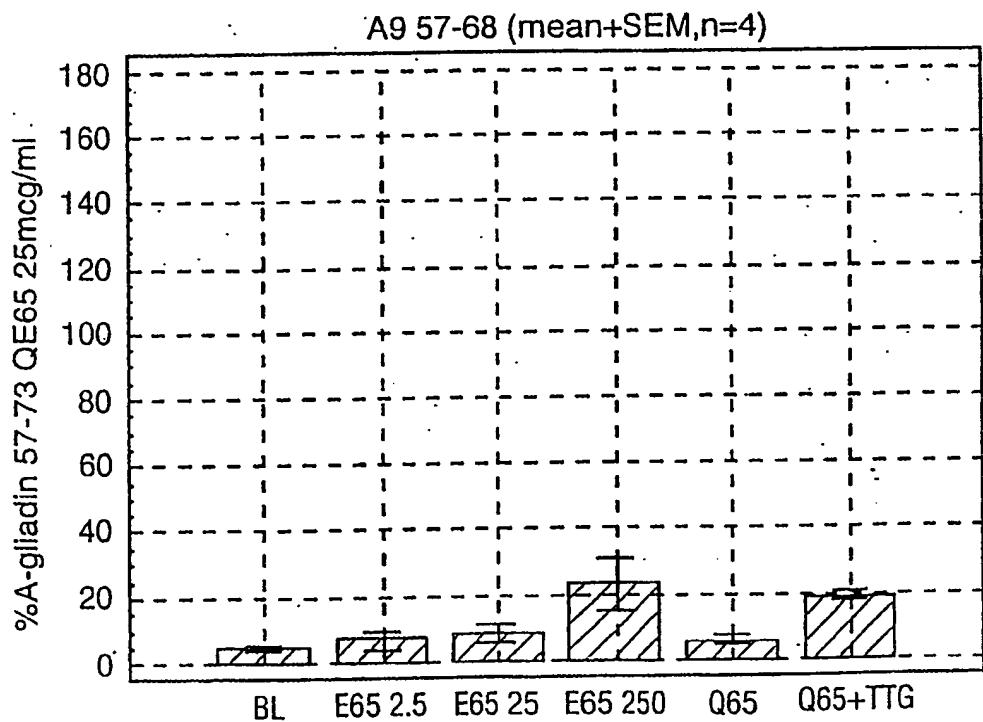


Fig.12f.

Dose response to the DQ2-restricted α gliadin T cell epitope α -2 62-75 QE65 & QE72: PQPELPYQPQPELPY (E65) (2.5, 25 & 250 mcg/ml), and α -2 62-75: PQPQLPYPQPQLPY (Q65) (25 mcg/ml) +/- tTG treatment.

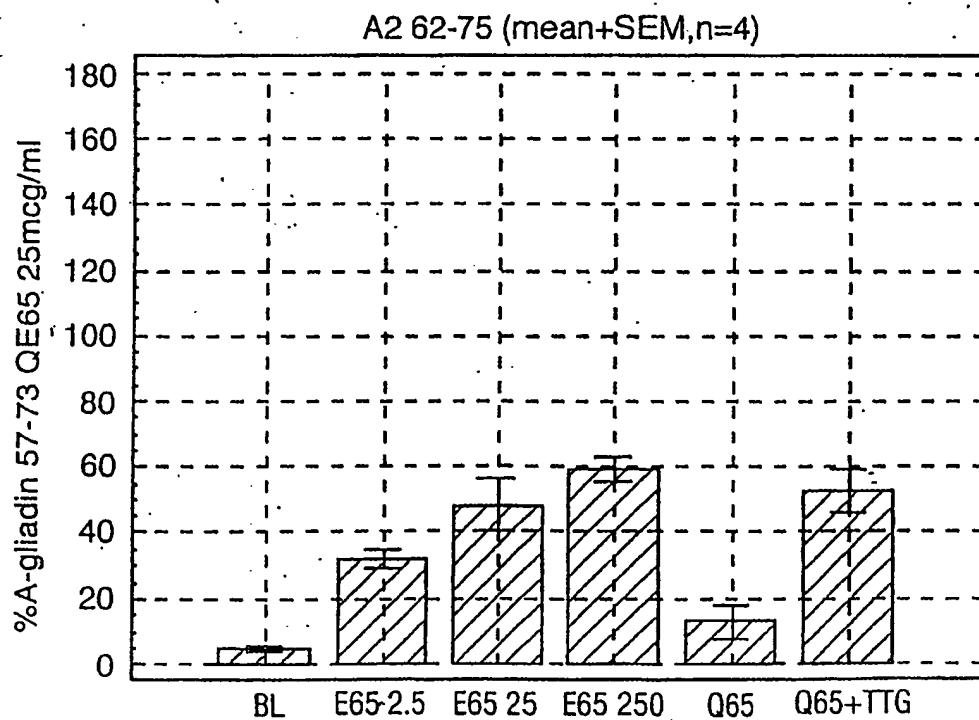


Fig.12g.

Dose response to the DQ8-restricted α gliadin T cell epitope GDA9 202-219: QE208 & 216: QQYPSGEGSFQPSQENPQ (E) (25 & 250 mcg/ml), and to GDA9 202-219 QQYPSGQGSFQPSQQNPQ (Q) (25 mcg/ml) +/- tTG treatment.

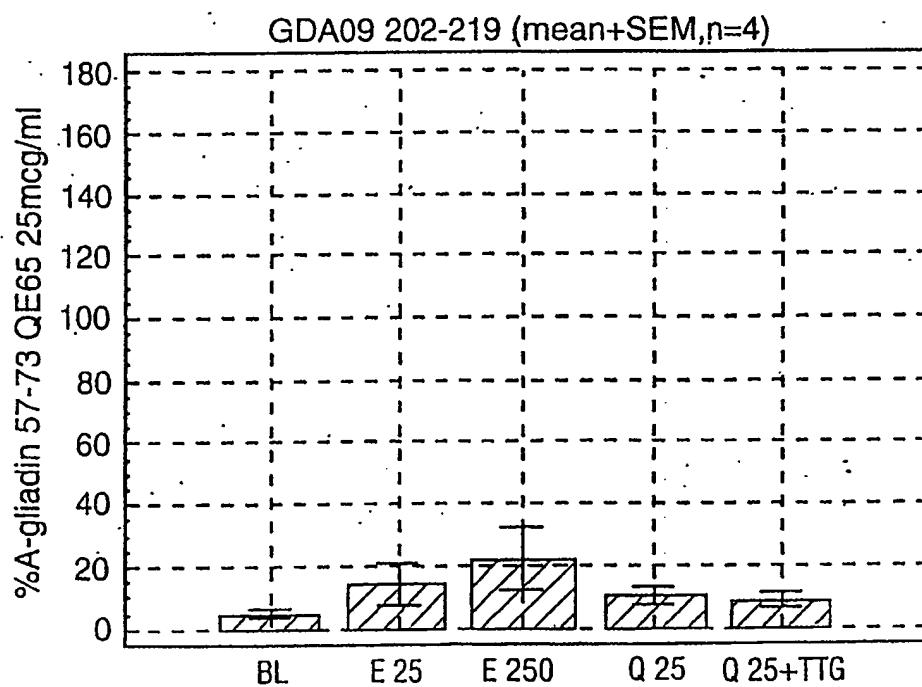


Fig.12h.

Dose response to the DQ2-restricted γ gliadin T cell epitope GDB2 134-153 QE140, 148,150: QQLPQPEQPQQSFPEQERPF (E) (25 & 250 mcg/ml), and to GDB2 134-153:

QQLPQPQQPQQSFPPQQQRPF (Q) (25 mcg/ml) +/- tTG treatment.

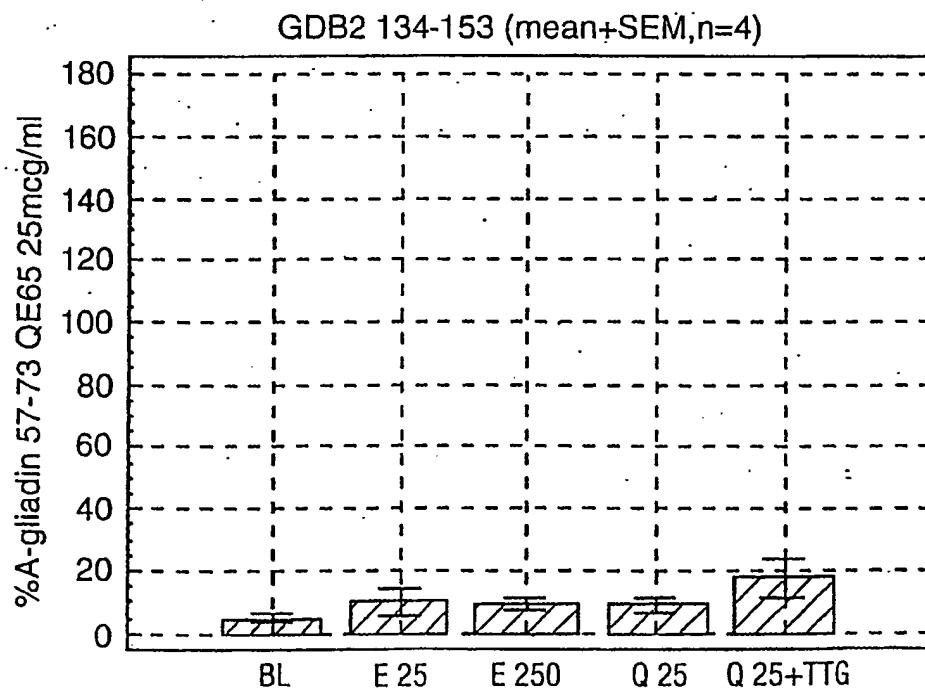
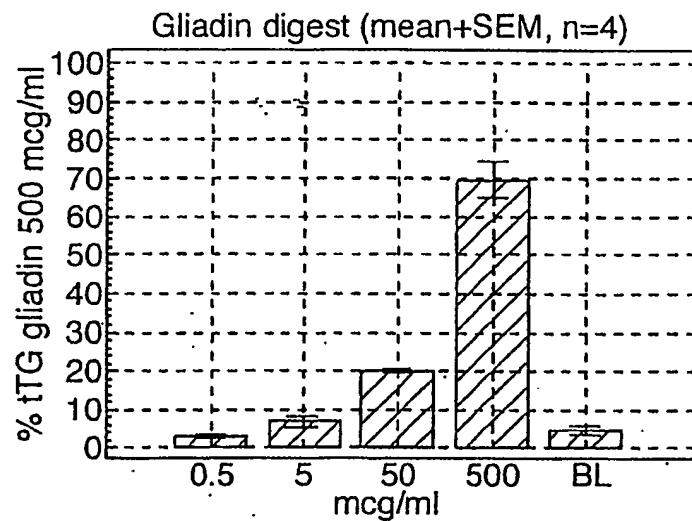


Fig.13a.

Dose response to gliadin digest by chymotrypsin.

**Fig.13b.**

Dose response to gliadin digested by chymotrypsin then treated with tTG.

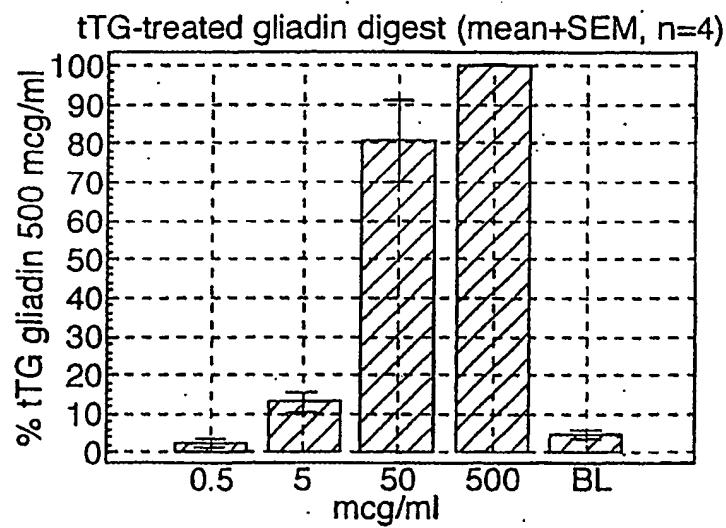
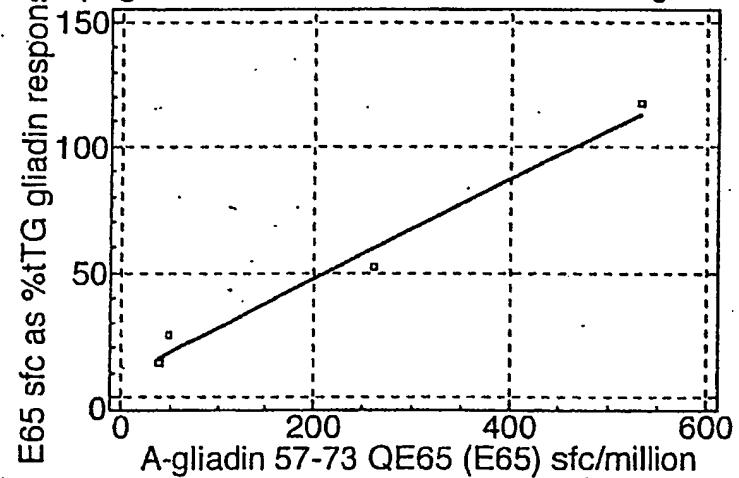


Fig. 13c.

Total ELISpot responses to A-gliadin 57-73 QE65 (25mcg/ml) versus A-gliadin 57-73 QE65 responses as percent of tTG gliadin (500mcg/ml) responses.

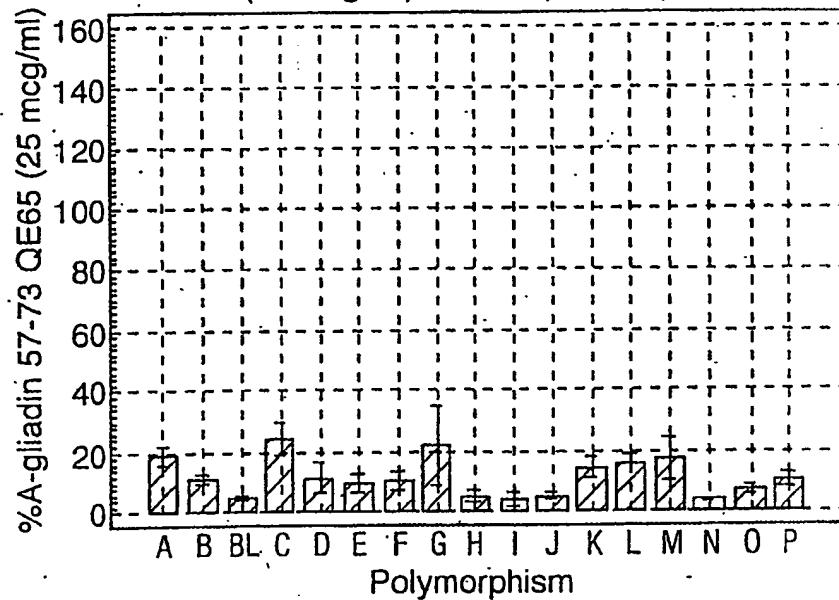
Responses to dominant epitope and complete antigen
(A-gliadin 57-73 QE65 and tTG-treated gliadin)



(Fig.14.)

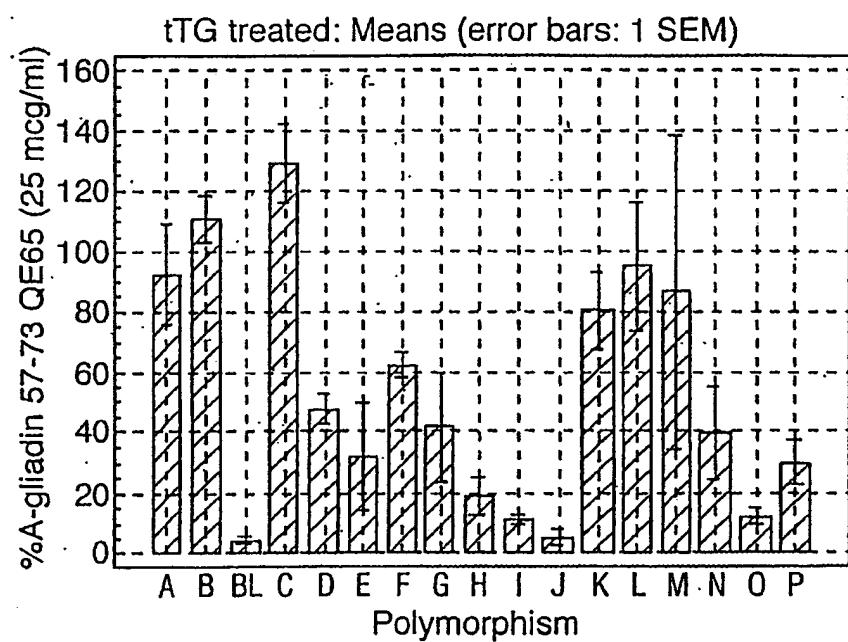
Bioactivity of gliadin polymorphisms of A-gliadin 57-73 (A) in coeliac subjects 6/7 days after gluten challenge (Gamma-Interferon Elispot) (n=4).

Fig.14a. Unmodified (25 mcg/ml): Means (error bars: 1 SEM)



A	QLQPFPQPQLPYPQPQS	I	QLQPFPQPQLSYSQPQP
B	QLQPFPQPQLPYPQPQP	J	QPQPFPQQPQLPYPQIQP
C	QLQPFPQPQLPYPQPQL	K	PQLPYPPQPQLPYPQPQP
D	QLQPFPQPQLPYLQPQS	L	PQLPYPPQPQLPYPQPQL
E	QLQPFPQPQLPYPQPQP	M	PQPQPFLPQLPYPQPQS
F	QLQPFPQPQLPYSQPQP	N	PQPQPFPQQPQLPYPQPQS
G	QLQPFLQPQLPYSQPQP	O	PQPQPFPQQPQLPYPQIQP
H	QLQPFSQPQLPYSQPQP	P	PQPQPFPQQPQLPYPQPPP

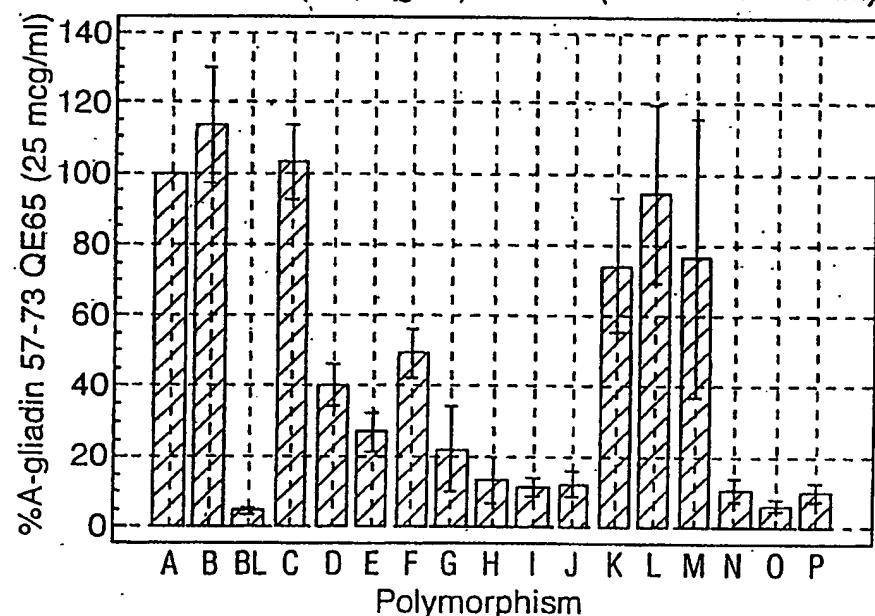
Fig.14b.



A	QLQPFPQPQLPYPQPQSQ	I	QLQPFPQPQLS <u>YS</u> QPQP
B	QLQPFPQPQLPYPQPQP	J	QPQPFP <u>PP</u> QLPYPQTQP
C	QLQPFPQPQLPYPQPQL	K	<u>PQL</u> PYPQPQLPYPQPQP
D	QLQPFPQPQLP <u>Y</u> LPQPQS	L	<u>PQL</u> PYPQPQLPYPQPQL
E	QLQPFP <u>R</u> PQLPYPQPQP	M	PQPQPFLPQLPYPQPQS
F	QLQPFPQPQLP <u>Y</u> SQPQP	N	PQPQPFP <u>PP</u> QLPYPQPQS
G	QLQPFLQPQLP <u>Y</u> SQPQP	O	PQPQPFP <u>PP</u> QLPYPQTQP
H	QLQPFS <u>Q</u> PQLP <u>Y</u> SQPQP	P	PQPQPFP <u>PP</u> QLPYPQP <u>PP</u>

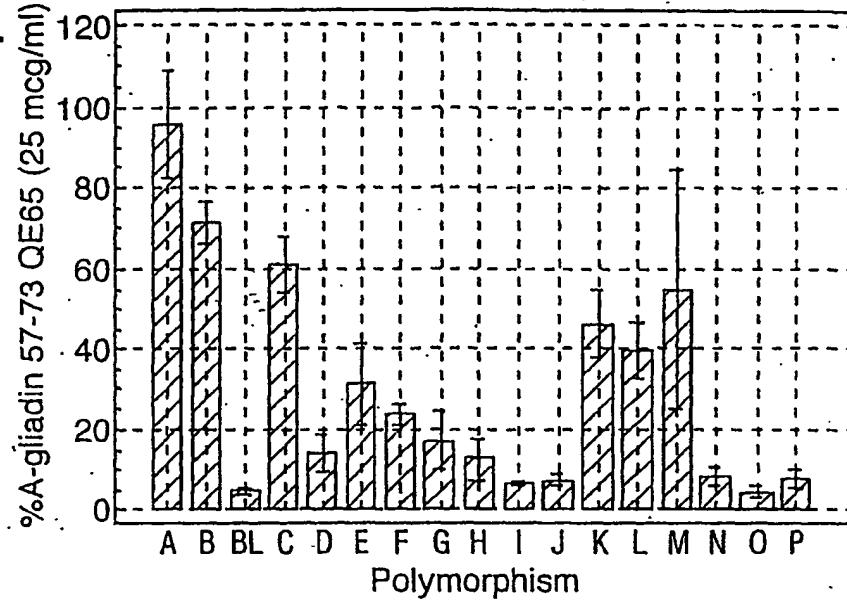
Fig.14c.

QE65 substituted (25 μg/ml): Means (error bars: 1 SEM)

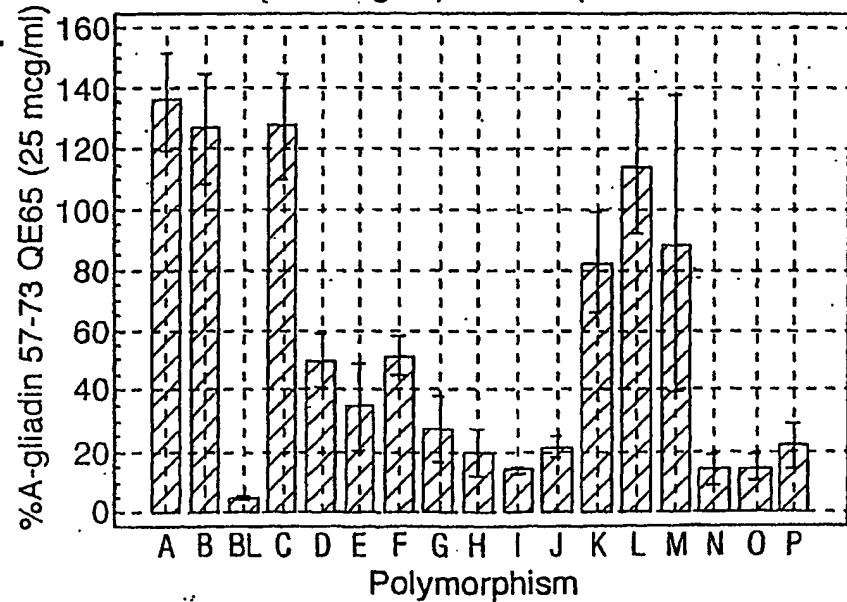


A	QLQPFPQPQLPYPQPQS	I	QLQPFPQPQLSYSQPQP
B	QLQPFPQPQLPYPQPQP	J	QPQPFP PP QLPYPQTQP
C	QLQPFPQPQLPYPQPQL	K	<u>PQL</u> PYPQPQLPYPQPQP
D	QLQPFPQPQLP Y LPQPQS	L	<u>PQL</u> PYPQPQLPYPQPQL
E	QLQPFP R PQLPYPQPQP	M	<u>PQPQF</u> LPQLPYPQPQS
F	QLQPFPQPQLPYSQPQP	N	<u>PQPQF</u> PPQLPYPQPQS
G	QLQPFLQPQLPYSQPQP	O	<u>PQPQF</u> PPQLPYPQTQP
H	QLQPFSQPQLPYSQPQP	P	<u>PQPQF</u> PPQLPYPQPPP

QE65-substituted (2.5 mcg/ml): Means (error bars: 1 SEM)
Fig.14d.



QE65-substituted (250 mcg/ml): Means (error bars: 1 SEM)
Fig.14e.



A QLQPFPQPQLPYPQPQPS
 B QLQPFPQPQLPYPQPQPS
 C QLQPFPQPQLPYPQPQL
 D QLQPFPQPQLPYLQPQS
 E QLQPFPBPQLPYPQPQPS
 F QLQPFPQPQLPYSQPQP
 G QLQPFLQPQLPYSQPQP
 H QLQPFSQPQLPYSQPQP

I QLQPFPQPQLSYSQPQP
 J QPQPFP~~PP~~QLPYPQTQP
 K PQLPYPQPQLPYPQPQP
 L PQLPYPQPQLPYPQPQL
 M PQPQF~~LP~~QLPYPQPQPS
 N PQPQFP~~PP~~QLPYPQPQPS
 O PQPQFP~~PP~~QLPYPQTQP
 P PQPQFP~~PP~~QLPYPQP~~PP~~

Fig.15.

Alanine scan: Means (error bars: 95% CI for mean)

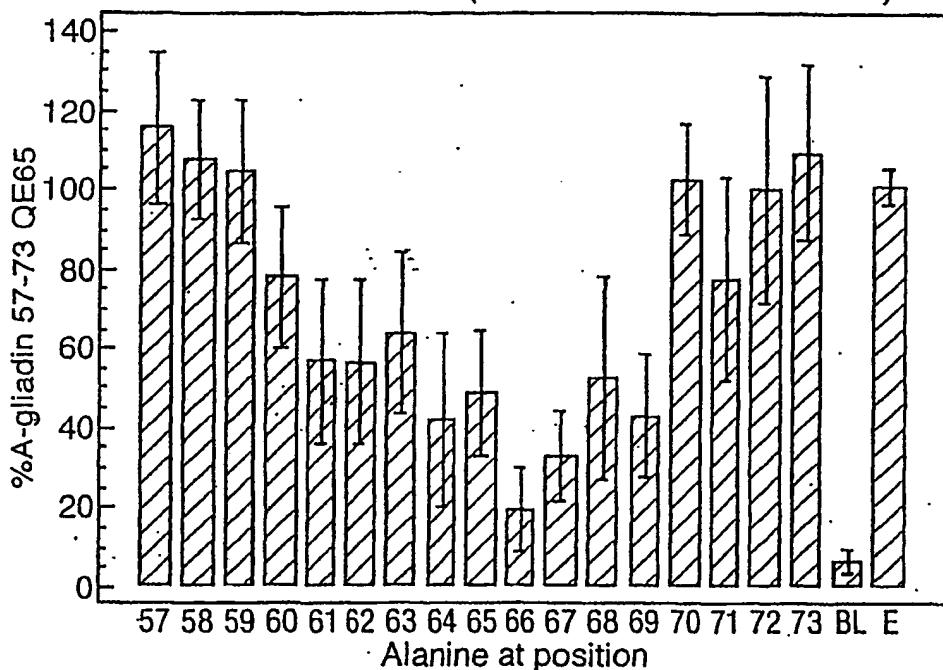


Fig.16.

Lysine scan: Means (error bars: 95% CI for mean)

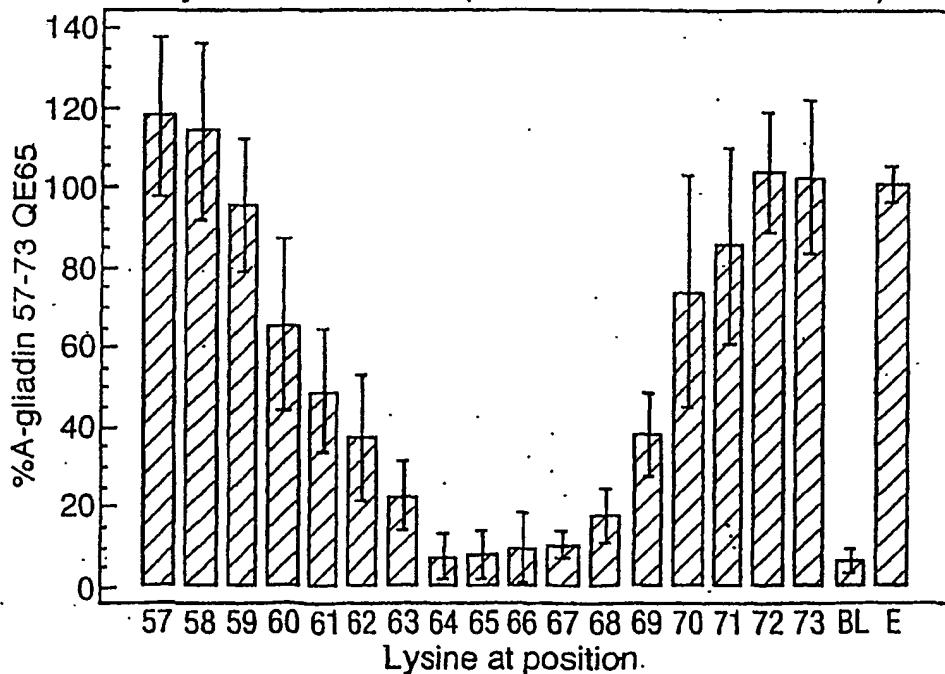


Fig.17.

Agonist activity of A-gliadin 57-73 QE65 variants according to position substituted (Mean of 8 coeliac subjects' PBMC responses in interferon gamma ELISPOT after gluten challenge)

QLQPFPQPELPYPQPQPS

60.....70

P60: Means (error bars: 95% CI for mean)

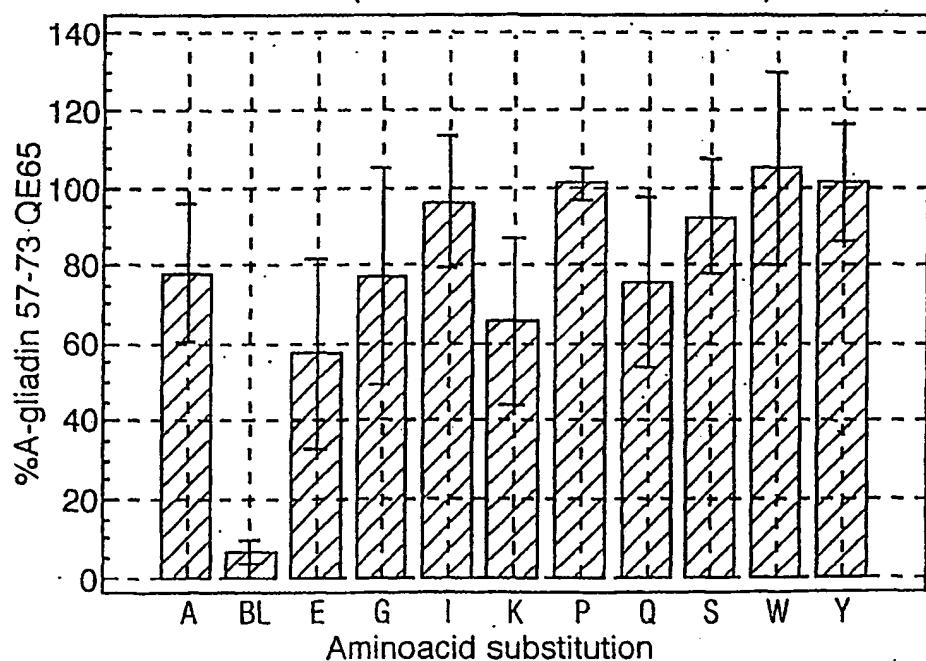


Fig.18.

Agonist activity of A-gliadin 57-73 QE65 variants according to position substituted (Mean of 8 coeliac subjects' PBMC responses in interferon gamma ELISPOT after gluten challenge)

QLQPFPQPELPYYPQPQPS

60.....70

F61: Means (error bars: 95% CI for mean)

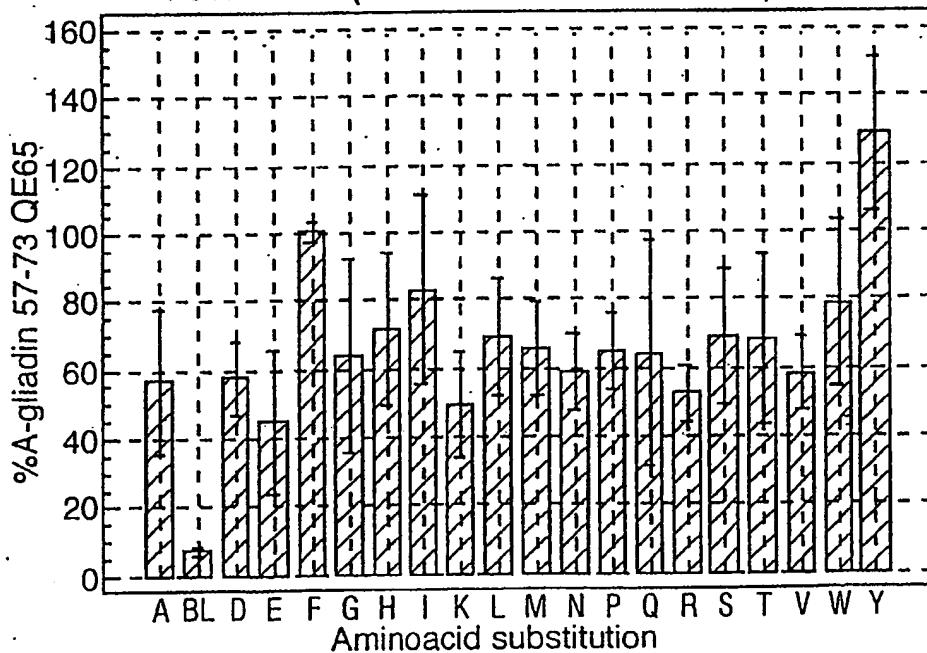


Fig.19.

Agonist activity of A-gliadin 57-73 QE65 variants according to position substituted (Mean of 8 coeliac subjects' PBMC responses in interferon gamma ELISPOT after gluten challenge)

$\text{QLQPFPQPPELPYPQPQPS}$
 60.....70

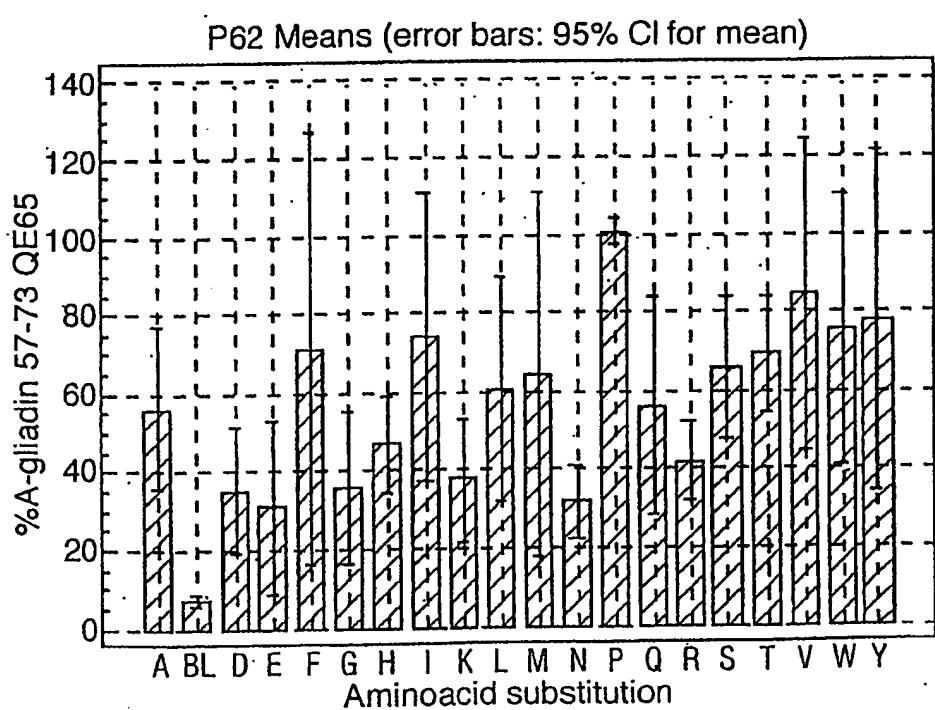


Fig.20.

Agonist activity of A-gliadin 57-73 QE65 variants according to position substituted (Mean of 8 coeliac subjects' PBMC responses in interferon gamma ELISPOT after gluten challenge)

QLQPFPQPELPYPQPQS

60.....70

Q63 Means (error bars: 95% CI for mean)

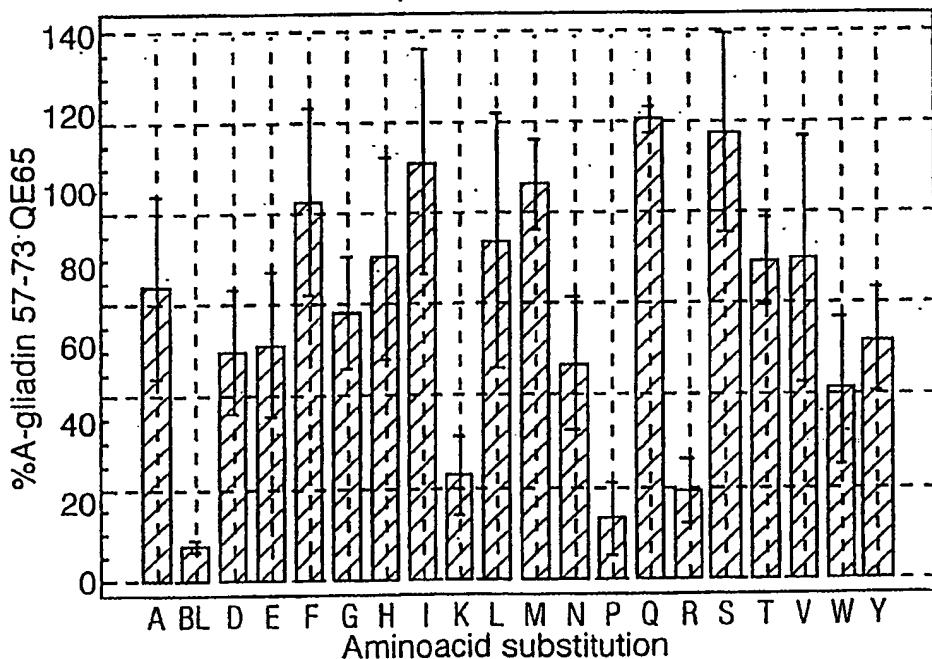


Fig.21.

Agonist activity of A-gliadin 57-73 QE65 variants according to position substituted (Mean of 8 coeliac subjects' PBMC responses in interferon gamma ELISPOT after gluten challenge)

QLQPFPQPELPYYPQPQS

60.....70

P64 Means (error bars: 95% CI for mean)

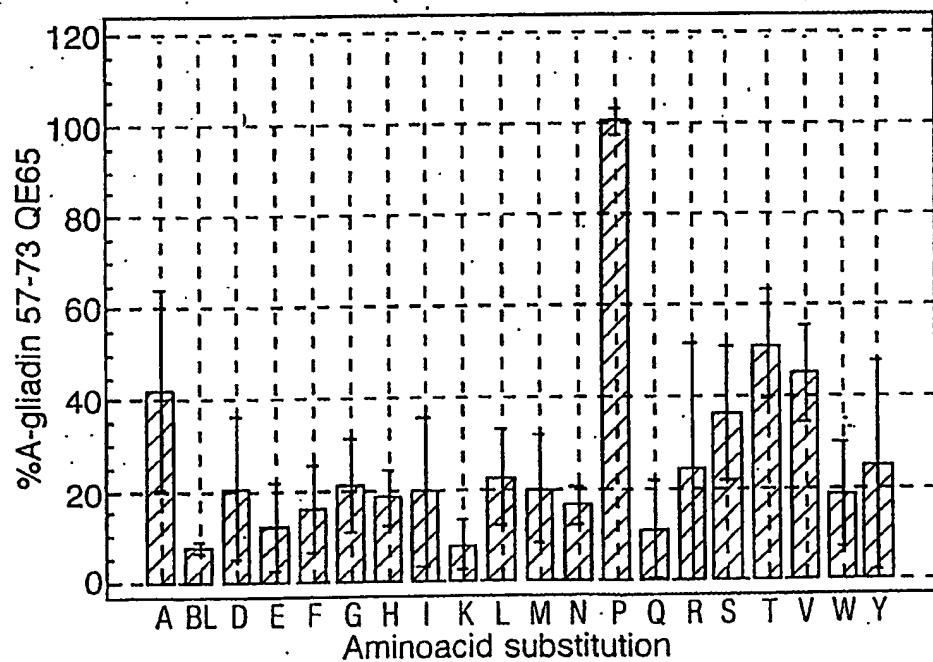


Fig.22.

Agonist activity of A-gliadin 57-73 QE65 variants according to position substituted (Mean of 8 coeliac subjects' PBMC responses in interferon gamma ELISPOT after gluten challenge)

QLQPFPQPELPYPQPQS

60.....70

E65 Means (error bars: 95% CI for mean)

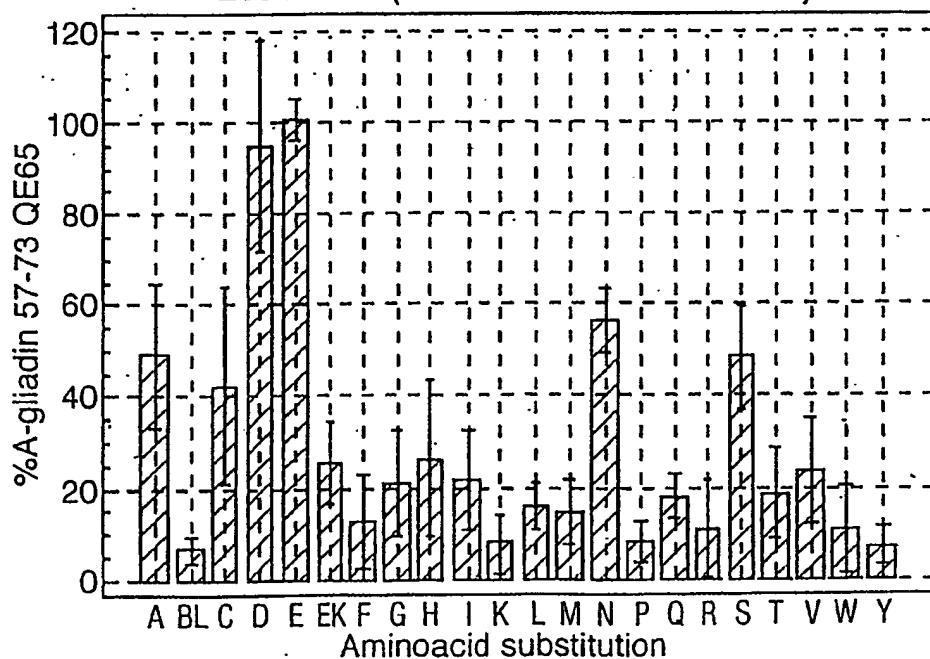


Fig.23.

Agonist activity of A-gliadin 57-73 QE65 variants according to position substituted (Mean of 8 coeliac subjects' PBMC responses in interferon gamma ELISPOT after gluten challenge)

QLQPFPQPELPYPPQPQS

60.....70

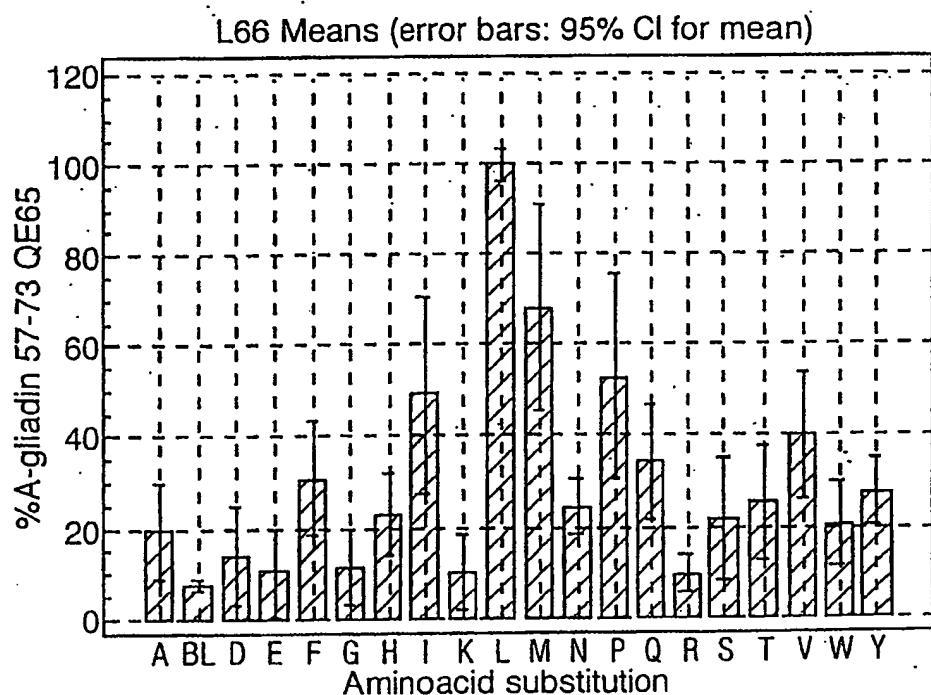


Fig.24.

Agonist activity of A-gliadin 57-73 QE65 variants according to position substituted (Mean of 8 coeliac subjects' PBMC responses in interferon gamma ELISPOT after gluten challenge)

QLQPFPQPELPYPQPQS

60.....70

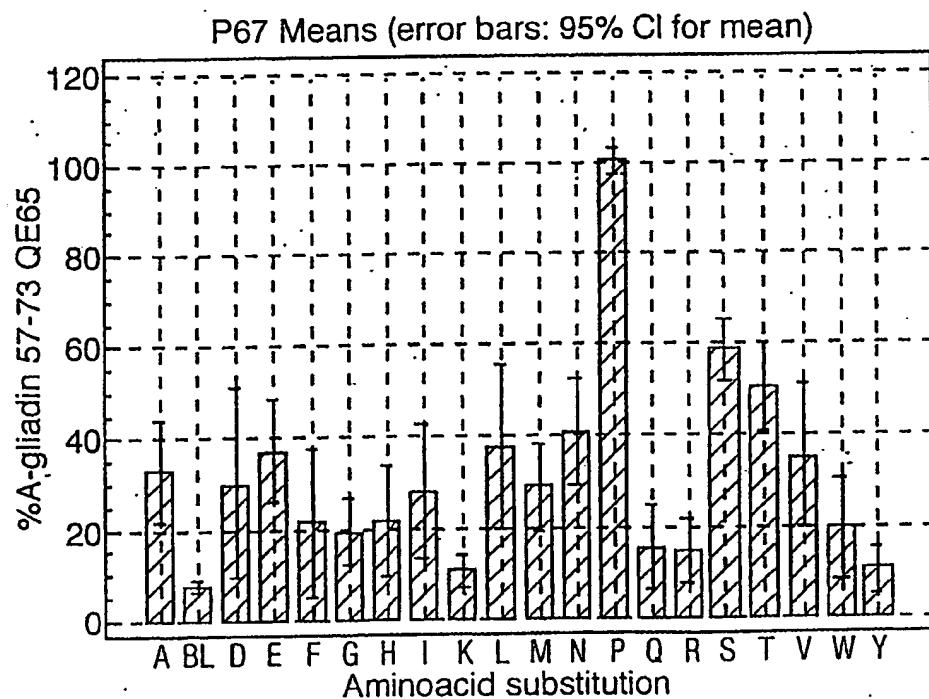


Fig.25.

Agonist activity of A-gliadin 57-73 QE65 variants according to position substituted (Mean of 8 coeliac subjects' PBMC responses in interferon gamma ELISPOT after gluten challenge)

QLQPFPQPPELPYPQPQS
60.....70

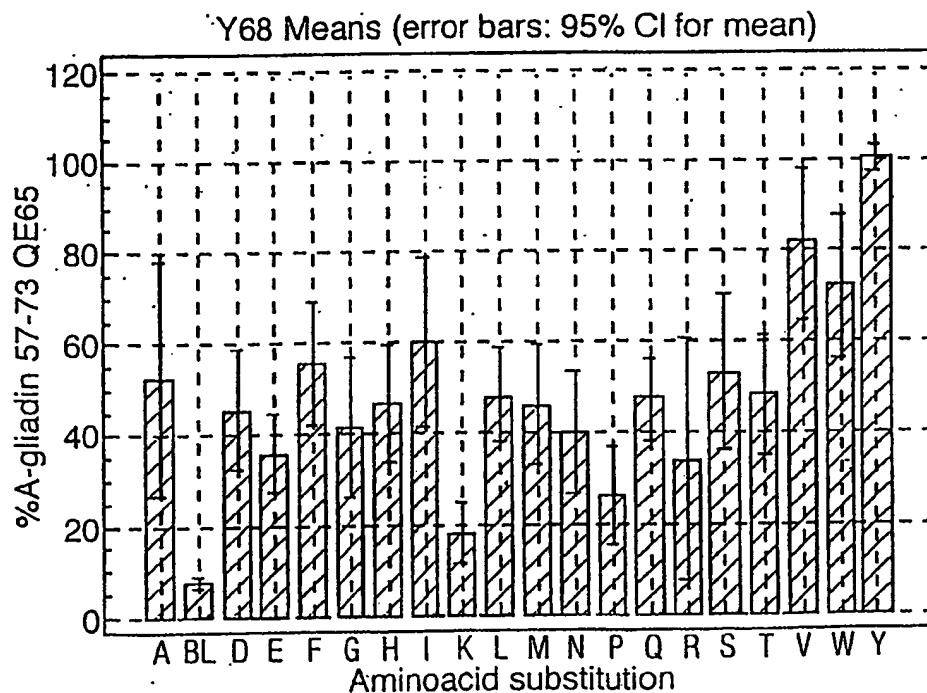


Fig.26.

Agonist activity of A-gliadin 57-73 QE65 variants according to position substituted (Mean of 8 coeliac subjects' PBMC responses in interferon gamma ELISPOT after gluten challenge)

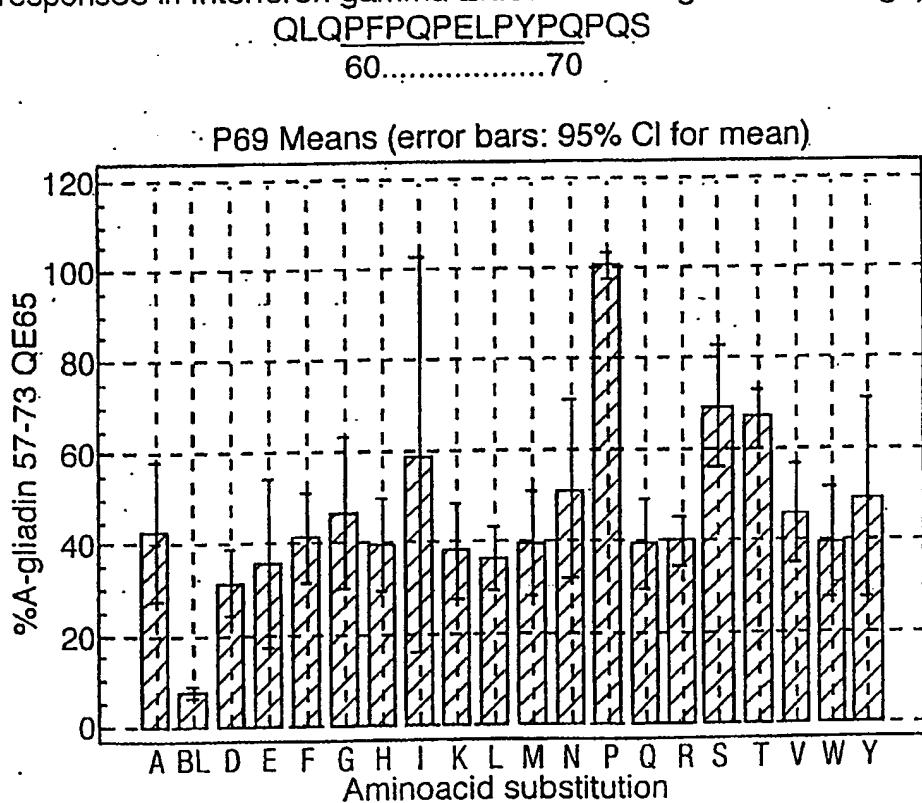


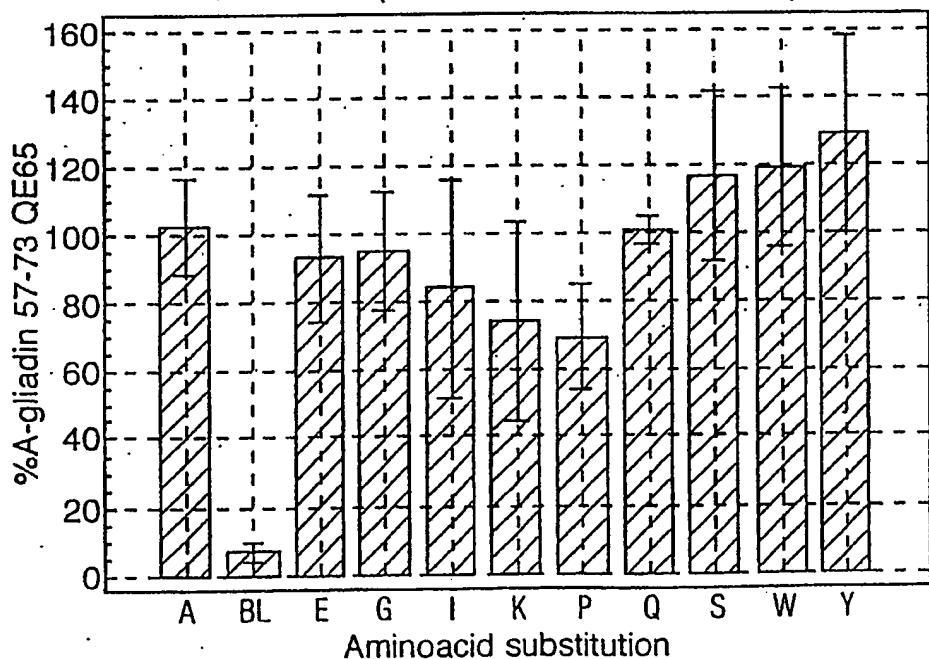
Fig.27.

Agonist activity of A-gliadin 57-73 QE65 variants according to position substituted (Mean of 8 coeliac subjects' PBMC responses in interferon gamma ELISPOT after gluten challenge)

QLQPFPQPELPYPQPQS

60.....70

Q70 Means (error bars: 95% CI for mean)



(Fig.28.)

Interferon gamma ELISpot responses in newly diagnosed and treated coeliac subjects, before and after gluten challenge.

Fig.28a. Untreated, newly diagnosed coeliacs (Mean+SEM, n=9)

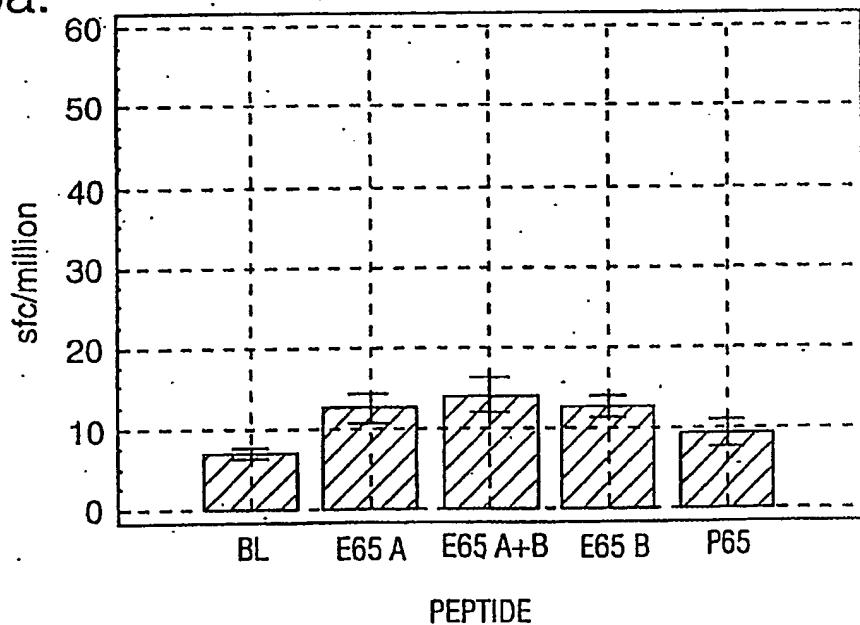


Fig.28b.

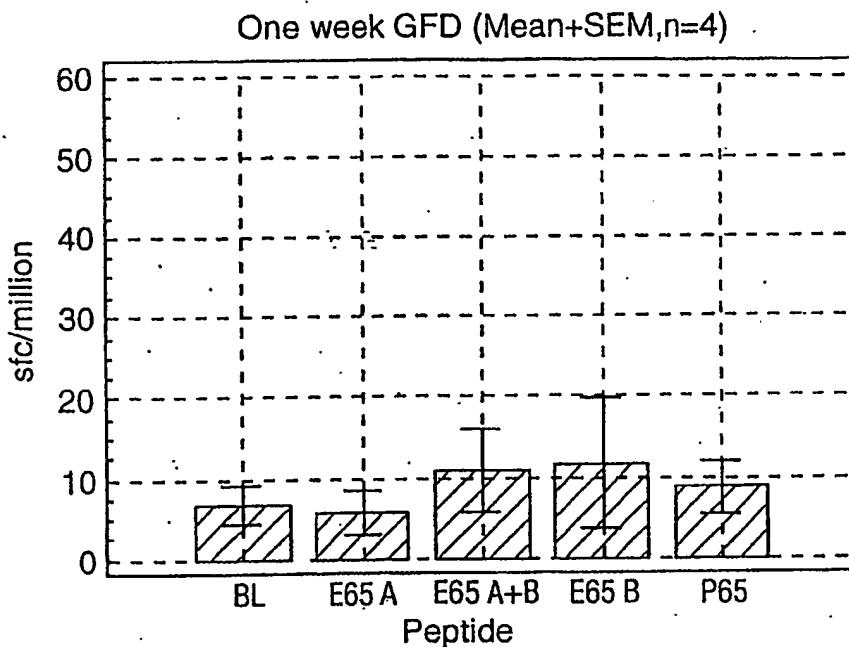


Fig.28c.

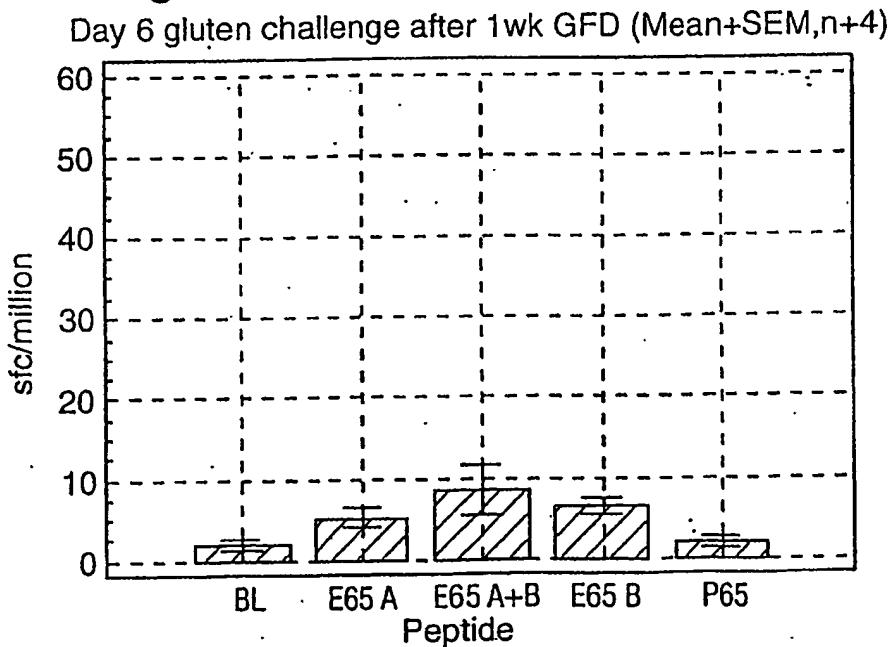


Fig.28d.

Two weeks GFD (Means+SEM,n=3)

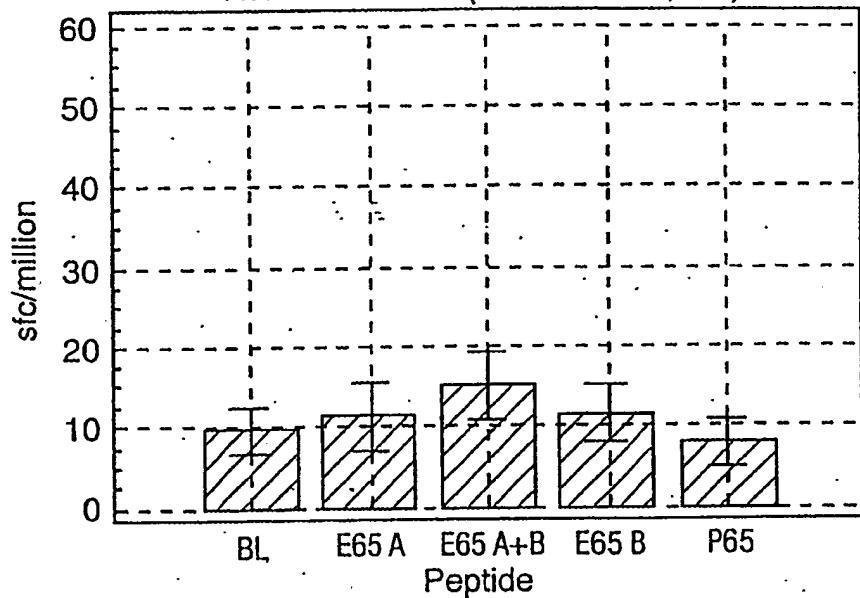


Fig.28e.

Day 6 gluten challenge after 2 wk GFD (Means+SEM,n=3)

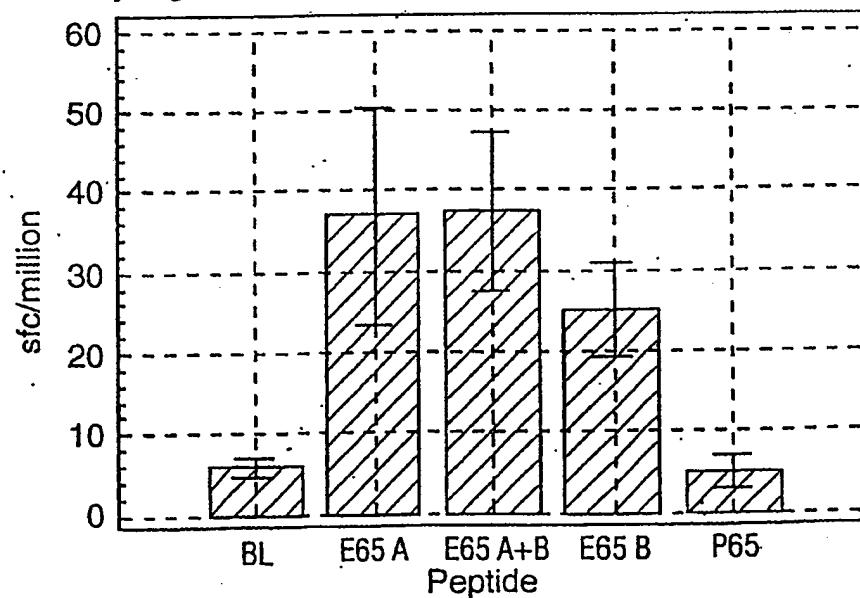


Fig.28f.

2 months GFD (Mean+SEM,n=3)

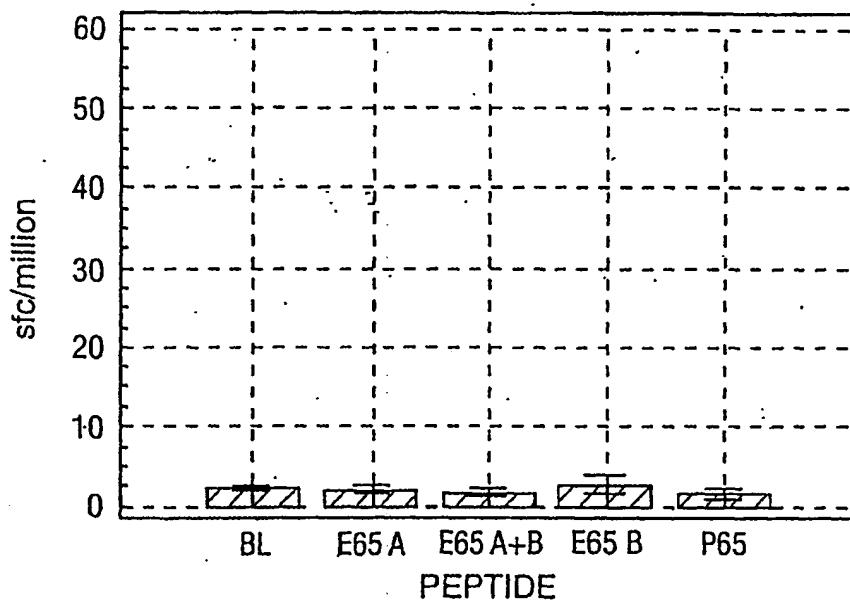


Fig.28g.

Day 6 gluten challenge after 2 mo GFD (Mean+SEM, n=3)

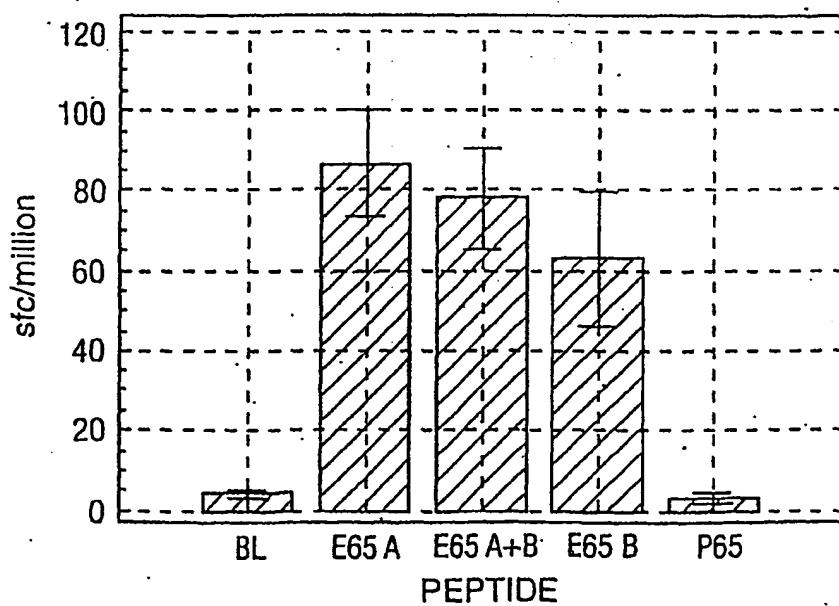
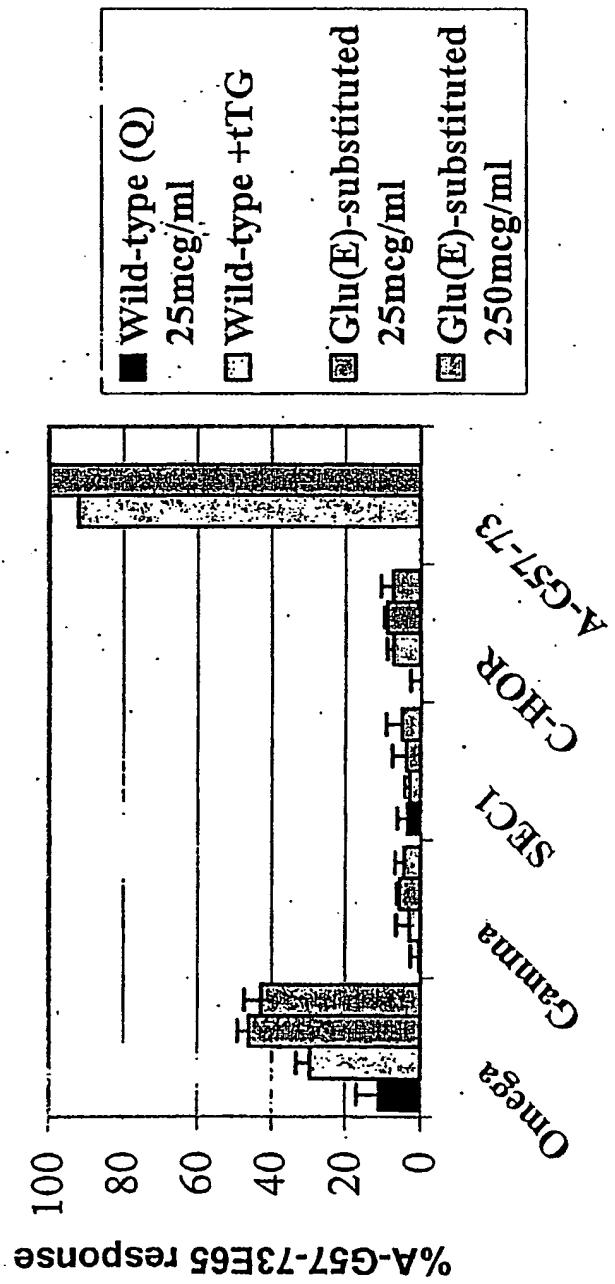


Figure 29. Bioactivity of prolamin homologues of α -gliadin 57-73 (IFNg-ELISpot, mean+SEM, n=6)



Omega: AAG17702 (141-157), Gamma: P21292 (96-112), SEC1: Q43639 (335-351), C-HOR: Q40055 (166-182). E-substituted peptides were synthesized with E for Q at position 9.

Figure 30. Healthy HLA-DQ2 Subjects: Change in IFNgamma ELISpot Responses to TTG-dexamidated Gliadin Peptide Pools (median change Day 6 vs Day 0, n=10).

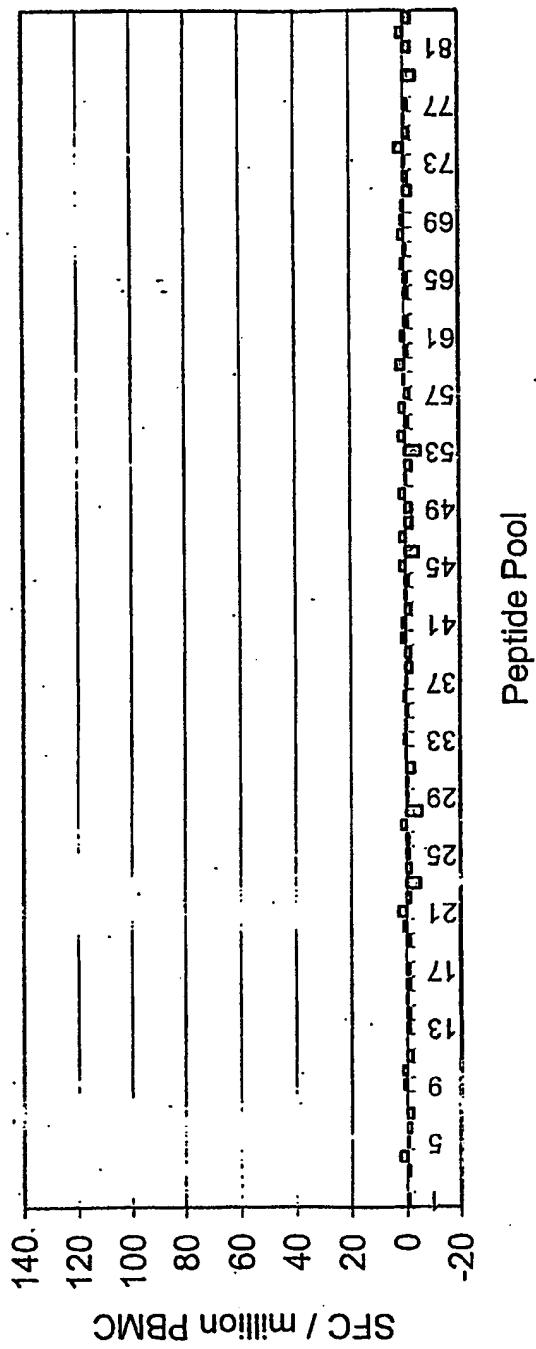


Figure 31. Coeliac HLA-DQ2 Subjects: Change in IFNgamma ELISpot Responses to tTG-deamidated Gliadin Peptide Pools (median change Day 6 vs Day 0, n=6)

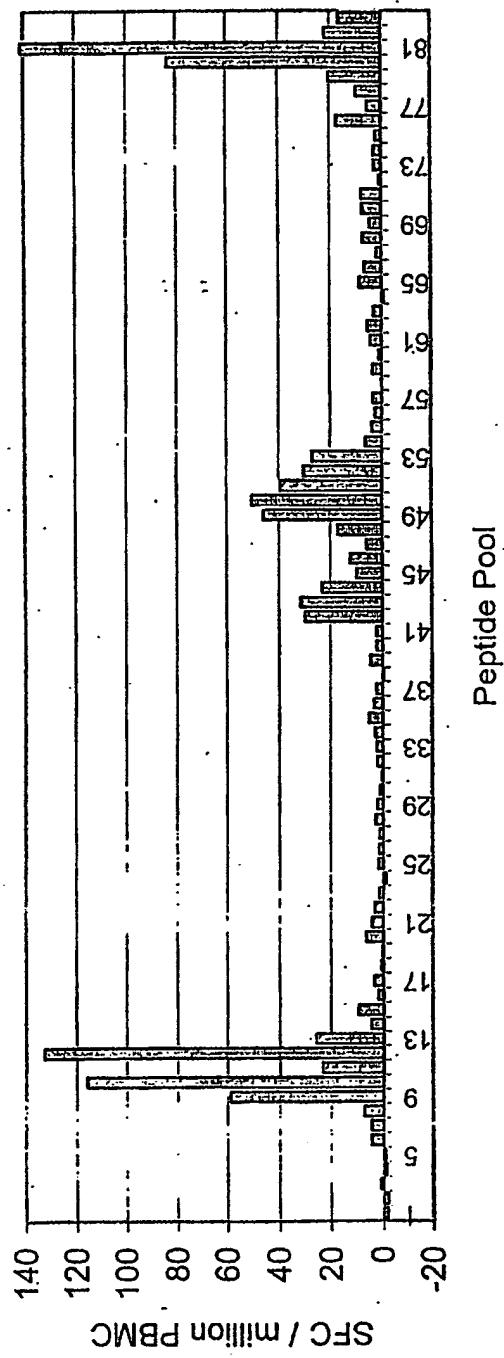


Figure 33. Coeliac HLA-DQ2/8 Subject C08: Gluten challenge induced IFNgamma ELISpot Responses to tTG-dreamidated Gliadin Peptide Pools

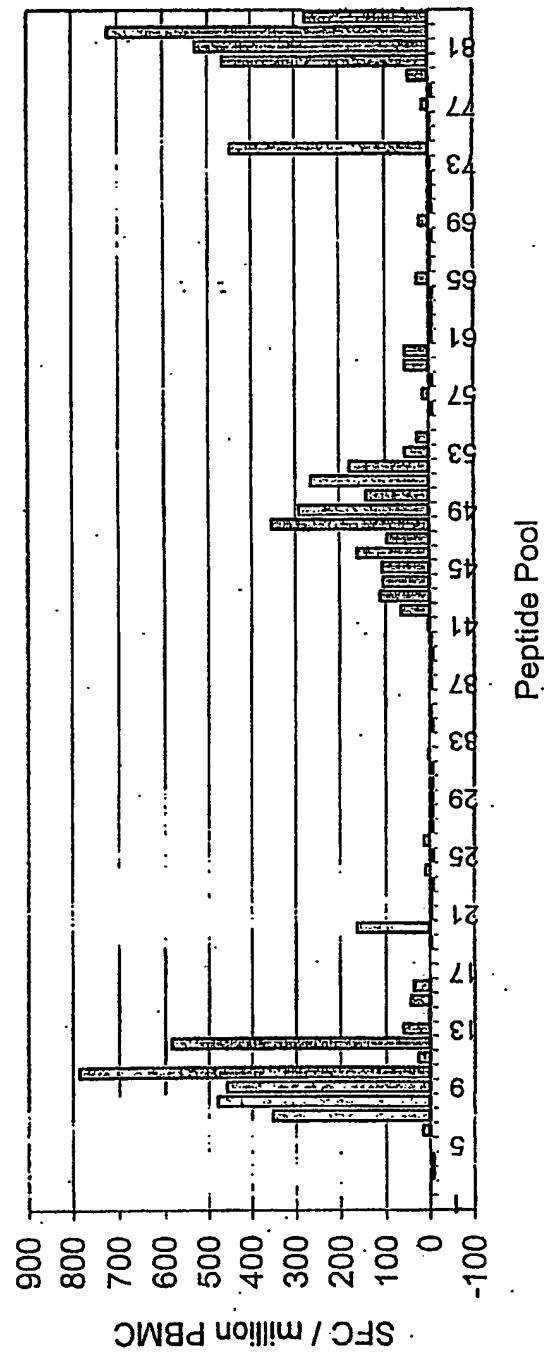


Figure 34. Coeliac HLA-DQ2/8 Subject C07: Change in IFNgamma ELISpot Responses to tTG-dleared Gliadin Peptide Pools

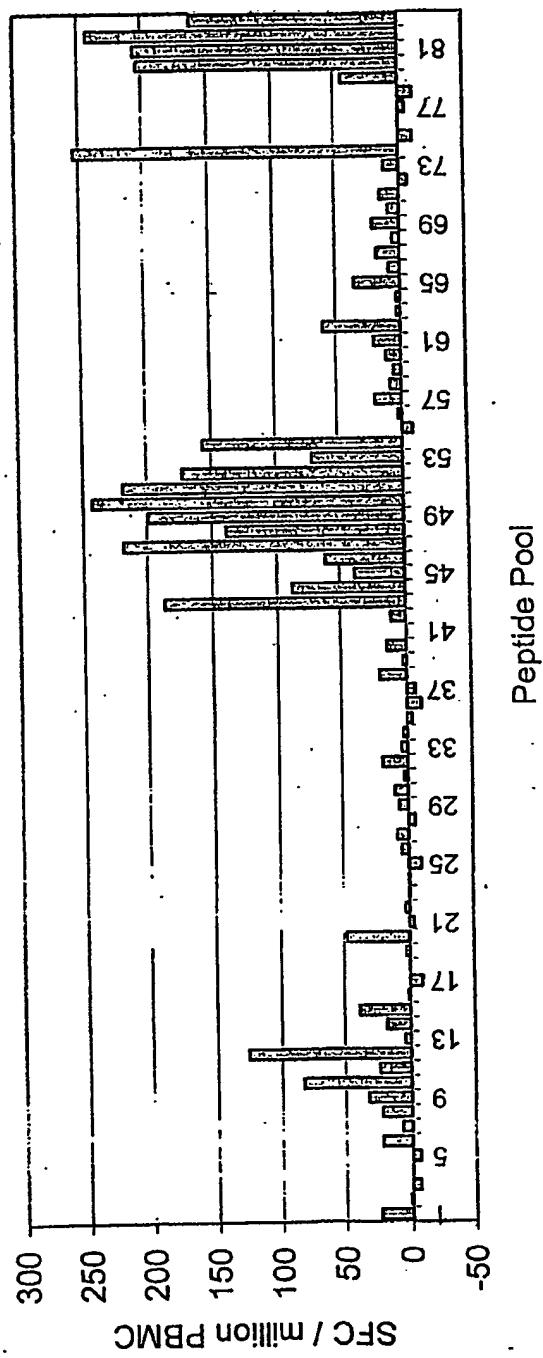


Figure 35. Coeliac HLA-DQ8/7 Subject C12: Gluten challenge induced IFNgamma ELISpot Responses to tTG-deamidated Gliadin Peptide Pools

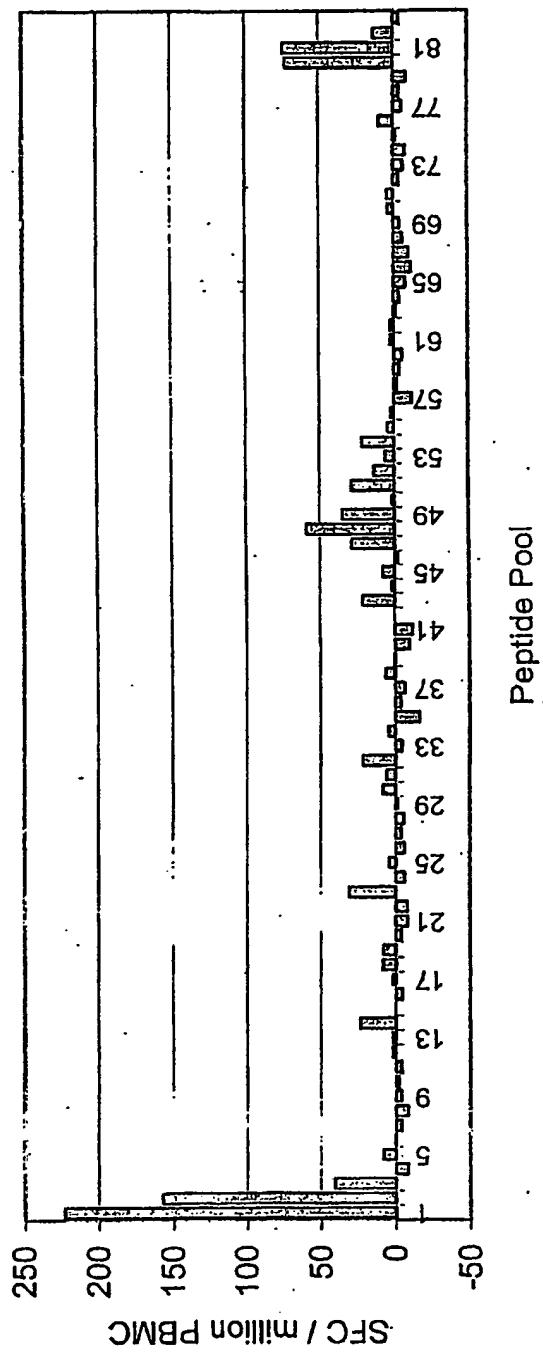
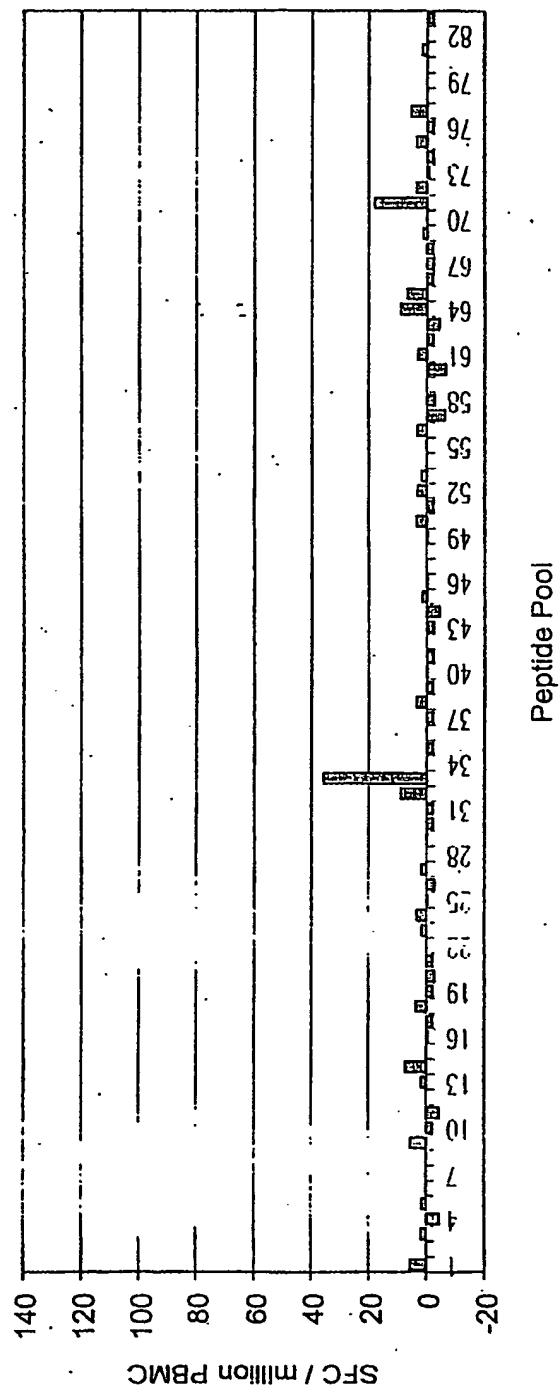


Figure 36. Coeliac HLA-DQ6/8 Subject C11: Change in IFNgamma ELISpot Responses to tTG-deamidated Gliadin Peptide Pools



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